European Chemicals Bureau

Institute for Health and Consumer Protection

European Chemicals Bureau

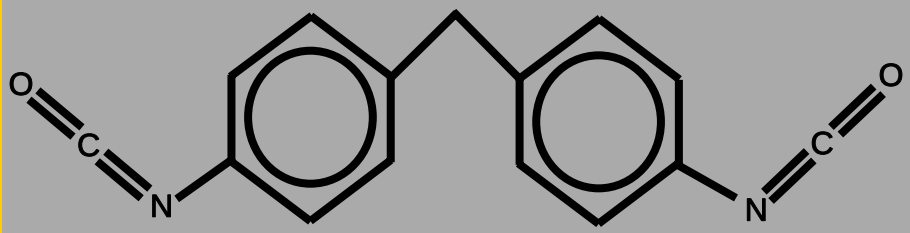
Existing Substances

European Union Risk Assessment Report

CAS No: 26447-40-5

EINECS No: 247-714-0

methylenediphenyl diisocyanate (MDI)



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European Union Risk Assessment Report

METHYLENEDIPHENYL DIISOCYANATE (MDI)

CAS No: 26447-40-5

EINECS No: 247-714-0

RISK ASSESSMENT

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METHYLENEDIPHENYL DIISOCYANATE (MDI)

CAS No: 26447-40-5

EINECS No: 247-714-0

RISK ASSESSMENT

Final Report, 2005

Belgium

The risk assessment of methylenediphenyl diisocyanate has been prepared by Belgium on behalf of the European Union.

Contact point:

BE Rapporteur: Federal Public Service Health, Food-Chain Safety and Environment
Environment Directorate-general
Mr. R. Moreau, General Director
Risk Management
Place Victor Horta, B 10
1060 Brussels
Contact point: linda.debacker@health.fgov.be

Scientific expertise:

Environmental part:

University of Liège
Laboratory of Animal Ecology and Ecotoxicology
Quai Van Beneden 22
B-4020 Liège
Belgium

Prof. J.P. Thomé
C. Joaquim-Justo

Human health part:

Katholieke Universiteit Leuven
Laboratorium voor Pneumologie
Eenheid voor Longtoxicologie
Herestraat 49 O & N
B-3000 Leuven
België

Prof. Dr. B. Nemery
H. Vanhooren

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Final report:	**2005**

Foreword

We are pleased to present this Risk Assessment Report which is the result of in-depth work carried out by experts in one Member State, working in co-operation with their counterparts in the other Member States, the Commission Services, Industry and public interest groups.

The Risk Assessment was carried out in accordance with Council Regulation (EEC) 793/93[1] on the evaluation and control of the risks of "existing" substances. "Existing" substances are chemical substances in use within the European Community before September 1981 and listed in the European Inventory of Existing Commercial Chemical Substances. Regulation 793/93 provides a systematic framework for the evaluation of the risks to human health and the environment of these substances if they are produced or imported into the Community in volumes above 10 t/year.

There are four overall stages in the Regulation for reducing the risks: data collection, priority setting, risk assessment and risk reduction. Data provided by Industry are used by Member States and the Commission services to determine the priority of the substances which need to be assessed. For each substance on a priority list, a Member State volunteers to act as "Rapporteur", undertaking the in-depth Risk Assessment and recommending a strategy to limit the risks of exposure to the substance, if necessary.

The methods for carrying out an in-depth Risk Assessment at Community level are laid down in Commission Regulation (EC) 1488/94[2], which is supported by a technical guidance document[3]. Normally, the "Rapporteur" and individual companies producing, importing and/or using the chemicals work closely together to develop a draft Risk Assessment Report, which is then presented at a Meeting of Member State technical experts for endorsement. The Risk Assessment Report is then peer-reviewed by the Scientific Committee on Toxicity, Ecotoxicity and the Environment (CSTEE) which gives its opinion to the European Commission on the quality of the risk assessment.

If a Risk Assessment Report concludes that measures to reduce the risks of exposure to the substances are needed, beyond any measures which may already be in place, the next step in the process is for the "Rapporteur" to develop a proposal for a strategy to limit those risks.

The Risk Assessment Report is also presented to the Organisation for Economic Co-operation and Development as a contribution to the Chapter 19, Agenda 21 goals for evaluating chemicals, agreed at the United Nations Conference on Environment and Development, held in Rio de Janeiro in 1992.

This Risk Assessment improves our knowledge about the risks to human health and the environment from exposure to chemicals. We hope you will agree that the results of this in-depth study and intensive co-operation will make a worthwhile contribution to the Community objective of reducing the overall risks from exposure to chemicals

Roland Schenkel
Acting Director-General
DG Joint Research Centre

Catherine Day
Director-General
DG Environment

[1] O.J. No L 084, 05/04/1993 p.0001 – 0075

[2] O.J. No L 161, 29/06/1994 p. 0003 – 0011

[3] Technical Guidance Document, Part I – V, ISBN 92-827-801 [1234]

0 OVERALL RESULTS OF THE RISK ASSESSMENT

CAS No:	26447-40-5
EINECS No:	247-714-0
IUPAC Name:	Methylenediphenyl diisocyanate

Environment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Human Health

Human Health (toxicity)

Workers

Conclusion (i) There is need for further information and/or testing

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (i) on hold is reached because:

- The current database does not adequately cover the toxicity for fertility. The collection of additional information should, however, not delay the implementation of appropriate control measures needed to address the concerns related to other endpoints (**conclusion (i) on hold**).

Conclusion (iii) is reached because:

- Health risks due to occupational exposure cannot be excluded with regard to irritation, both skin and eyes, for unprotected workers on building sites.
- Health risks due to occupational exposure cannot be excluded with regard to respiratory tract irritation.
- Health risks due to occupational exposure cannot be excluded with regard to sensitisation (dermal contact and inhalation exposure).
- Health risks due to occupational exposure cannot be excluded with regard to repeated inhalation exposure.

Consumers

Conclusion (i) There is need for further information and/or testing

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (i) on hold is reached because:

- The current database does not adequately cover the toxicity for fertility. The collection of additional information should, however, not delay the implementation of appropriate control measures needed to address the concerns related to other endpoints (**conclusion (i) on hold**).

Conclusion (iii) is reached because:

- Risk reduction measures should be considered that will ensure protection of consumers from eye and skin and respiratory tract irritation, respiratory and skin sensitisation, and lung effects induced by short-term repeated exposure.

Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Combined exposure: workers/consumers/humans exposed via the environment

Conclusion (i) There is need for further information and/or testing

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (i) on hold is reached because:

- The current database does not adequately cover the toxicity for fertility. The collection of additional information should, however, not delay the implementation of appropriate control measures needed to address the concerns related to other endpoints (**conclusion (i) on hold**).

Conclusion (iii) is reached because:

- Health risks due to combined occupational and consumer exposure cannot be excluded with regard to irritation (eyes, skin, respiratory tract).
- Health risks due to combined occupational and consumer exposure cannot be excluded with regard to sensitisation (dermal contact and inhalation exposure).
- Health risks due to combined occupational and consumer exposure cannot be excluded with regard to repeated inhalation exposure.

<u>Human Health (risks from physico-chemical properties)</u>

Conclusion (ii) There is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Conclusion (ii) is reached because:

- No concern is expected related to the physico-chemical properties of the substance for human populations (workers, consumers and humans exposed via the environment).

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1 GENERAL SUBSTANCE INFORMATION

The term 'MDI' represents a number of isomeric compounds with the empirical formula $C_{15}H_{10}N_2O_2$, as well as prepolymers and polymers based on these isomers.

The material defined by EINECS 247-714-0 and CAS 26447-40-5, by not specifying isomeric forms, encompasses not only isomeric mixtures but also all of the specific isomers even if those isomers have a specific CAS or EINECS number.

The possible isomeric forms of MDI are:

4,4'-methylenediphenyl diisocyanate	EINECS No 202-966-0 CAS 101-68-8
2,4'-methylenediphenyl diisocyanate	EINECS No 227-534-9 CAS 5873-54-1
2,2'-methylenediphenyl diisocyanate	EINECS No 219-799-4 CAS 2536-05-2

However, industrial production of MDI does not produce pure isomers, but a mixture primarily containing 4,4'-isomer, with isomer proportions varying according to the exact process used.

Monomeric 4,4'-MDI is produced only by distillation of the isomer mixture, and the 2,4'-and 2,2'-isomers are very difficult to isolate.

Hence, the production material, being of mixed and variable proportion of isomers, and by common usage given the name generic MDI, and also non-isomer specific MDI, is best defined by CAS 26447-40-5, as this does not specify any particular isomer or proportion of isomers in the product.

Data gained on any mixture of MDI isomers, regardless of relative isomer proportions, as well as on any individual isomer, are considered representative for the purpose of hazard evaluation and risk assessment. The term MDI in this report is taken as the generic name referring to any of these isomeric substances and mixtures (and preparations) unless specified.

Assessing the (eco)toxicity of MDI is very complicated. Testing the pure isomers is difficult because isolation of the pure isomers, 2,4' and 2,2' MDI, is technologically challenging and the physical state of the pure 4,4' MDI necessitates the use of interfering solvents in (eco)toxicity tests. Furthermore, because of the extreme reactivity of the MDI - with its intrinsically poor solubility - with water, most of the tests are thus difficult to perform.

A summary of information on registered substances falling under the general designation of 'MDI', and their classification and labelling is given **Figure 1.1**.

1.1 IDENTIFICATION OF THE SUBSTANCE

CAS No:	26447-40-5
EINECS No:	247-714-0
IUPAC Name:	Methylene bis (phenyl isocyanate) (US-EPA, 1994), Methylenediphenyl diisocyanate
Synonyms:	MDI (common name), 4,4'-diphenyl methane diisocyanate,2,4'-diphenyl methane diisocyanate, 2,2'-diisocyanatodiphenylmethane,

	Methylene bisphenyl isocyanate, Crude MDI, Polymeric MDI, PMDI, Generic MDI, Non isomer specific MDI
Structural formula:	see **Figure 1.1**
Molecular formula:	$C_{15}H_{10}N_2O_2$ (monomeric), $C_{15}H_{10}N_2O_2$. $[C_8H_5NO]_n$, (polymeric)
Molecular weight:	Monomeric MDI: 250.26 (Dow, 1986a Polymeric MDI: 290-400 (Dow, 1986b)

1.2 PURITY/IMPURITIES, ADDITIVES

Composition of the substance:

4,4'-MDI	4,4'-MDI	> 97%
	2,4'-MDI	1.5-2.5%
	2,2'-MDI	> 0.5%
Polymeric MDI	4,4'-MDI	40-50%
	2,4'-MDI	2.5-4.0%
	2,2'-MDI	0.1-0.2%
	Homologues	60-50%

The substance is marketed as Polymeric MDI or 4,4'-MDI. Trade names of the product from the various producers are:

BASF	Lupranate
Bayer	Desmodur
Dow	Voranate
Dow	Tedimon
Huntsman	Suprasec
Shell	Caradate

The concentration of MDI in the marketed product depends on the application considered and can vary from non detectable to 100% (for example, adhesive and lacquers to pure MDI used in elastomers).

1.2.1 Purity

- > 97% w/w (Annex VIIA doc., EU Questionnaire: from Industry)

1.2.2 Impurities

- monochlorobenzene (max. 80 ppm: Annex VIIA)
- phenylisocyanate (max. 50 ppm: AnnexVIIA)
- hydrogen chloride

1.2.3 Additives

Any of the following compounds may be used as stabiliser:

- triphenyl phosphite (TPP)
- dinonyl phthalate (DNP)
- triethyl phosphate (TEP)
- butyl hydroxy toluene (BHT)

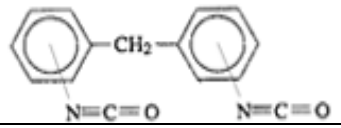
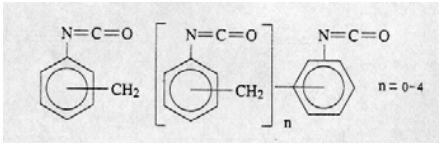
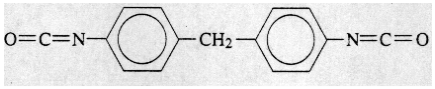
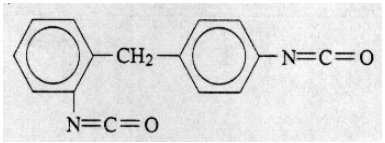
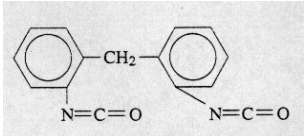
- Concentration range 200 – 1,000 ppm (Annex VIIA)

1.3 PHYSICO-CHEMICAL PROPERTIES

Collected and published data report mainly on the 4,4'- and the polymeric MDI. Few testing results are available on the 2,4' MDI.

Table 1.1 summarises the chemical and physical properties of MDI. Some more information is given in the following Sections (see also IUCLID).

Table 1.1 MDI and EU terminology and regulations

Identification numbers	EINECS name *(IUPAC name)*	Structural formula	Entry in Annex I of the Reg. 793/93/EC	Entry in Annex I of the Dir. 67/548/EEC
EINECS nr: 247-714-0 CAS-nr: 26447-40-5	Benzene 1,1'-methylenebis (isocyanato- *(Methylenediphenyl diisocyanate)*		yes	yes (28h ATP, i.e. Dir. 2001/59/EC, O.J. 21. 08.2001)
EINECS-nr: none CAS-nr: 9016-87-9			n.a.	no
EINECS-nr: 202-966-0 CAS-nr: 101-68-8	Benzene, 1,1'-methylenebis (4-isocyanato- *(4,4'-methylenediphenyl diisocyanate)*		no	yes (Dir. 98/98 = 25th ATP) Index nr 615-005-00-9
EINECS-nr: 227-534-9 CAS-nr: 5873-54-1	Benzene, 1-isocyanato-2-((4-isocyanatophenyl)methyl)- *(2,4'-methylenediphenyl diisocyanate)*		no	yes (Dir. 98/98 = 25th ATP) Index nr 615-005-00-9
EINECS-nr: 219-799-4 CAS-nr: 2536-05-2	Benzene, 1,1'-methylenebis (2-isocyanato- *(2,2'-methylenediphenyl diisocyanate)*		no	yes (Dir. 98/98 = 25th ATP) Index nr 615-005-00-9
NLP-nr: 500-040-3 CAS-nr: 25686-28-6	NLP-name: 4,4'-methyldiphenyl diisocyanate, oligomers (max. 3 benzene rings)		n.a.	no

1.3.1 Physical state (at NTP)

Polymeric MDI is a dark amber viscous liquid while the pure 4,4' MDI is a white waxy solid. The odour of MDI is slightly musty.

1.3.2 Melting point

The melting point depends upon the nature of the manufactured material.

A value of 5°C has been validated for polymeric MDI containing 50% 4,4' MDI (Shell, 1994a). Below 10°C crystals are formed.

For monomeric 4,4' MDI a melting point of 39 to 43°C has been determined by EU standard method A1 under GLP (Shell, 1994b) while for the 2,4' isomer the range of 34 - 38°C was detected under the same standards (EC test, capillary method and compliance to GLP; Shell 1994c).

1.3.3 Boiling point

Studies suggest that some thermal decomposition/polymerisation occurs at approximately 230°C for 4,4' MDI and 358°C for polymeric MDI before the boiling point is reached (Dow, 1986a and b).

A study conducted in compliance with GLP and following the EU standard method A2 provided a boiling point of >300°C for 4,4' MDI and polymeric MDI when the Siwoloboff method is used; the differential scanning calorimetry method yielded boiling points of respectively 364°C and >358°C for 4,4' MDI and polymeric MDI (Shell, 1994 b and a) quoted 364°C at 101325 Pa and >358°C at 101,100 Pa respectively for the 4,4' isomer and the polymeric form in EC standard testing and under GLP. In general, for 4,4' MDI and polymeric MDI the value > 300°C at approximately 101,100 Pa is quoted as valid (Annex VIIA).

1.3.4 Relative density

The relative density measured according to the EU standard method A3 at 20°C has been quoted at 1.325 (4,4' MDI) to 1.2381 (polymeric MDI)(Shell, 1994a and b). Other values are available in the IUCLID data set.

1.3.5 Vapour pressure

The following vapour pressures reported from tests conducted in compliance with GLP (EC method A4, static procedure) were accepted: 0.0014; < 0.002 and < 0.005 Pa at 20°C respectively for 2,4' MDI (Shell, 1994c); 4,4' MDI (Shell, 1994b) and polymeric MDI (Shell, 1994a). The highest of these values was used for modelling purposes.

1.3.6 Solubility

Determination of the MDI solubility in water is difficult because of the high reactivity of the NCO groups it contains towards OH groups. Consequently it is not possible to measure the solubility of MDI in water using the EC standard methods.

In the III project FE-E-93 (III, 1997), Yakabe measured the log P_{ow} of generic MDI by the HPLC method and he calculated the water solubility of MDI using different equations that link the log P_{ow} and the log water solubility. The calculated values varied between 0.0201 and 1.39 mg/l. The value used for model calculation was 0.0201 mg/l. Even if these values have little or no relevance to risk assessment modelling given the high reactivity of the substance with water, they are very important to understand the behaviour of the substance in the environment when it is in contact with water. Yakabe also analysed the residual amount of generic MDI present in water when increasing concentrations of MDI dissolved in acetonitrile were added in water; since no residual amount of MDI was detected when a concentration of 0.03 mg of MDI was added per litre of water it was considered that this value could be equated to the water solubility of the studied substance.

All values obtained are probably much higher than the achievable concentrations when adding MDI to water. Nevertheless, in the environmental risk assessment the water solubility of MDI was considered to be 0.02 mg/l; this value was taken as a worst case value.

1.3.7 Octanol-water partition coefficient

The log (octanol-water) partition coefficient of 4.5 was measured by HPLC (method OECD n° 117) for generic MDI (III, 1997). However, this value was judged as “not relevant” for predicting the bioconcentration factor in aquatic species, since dissolved MDI has only a transient existence in water and, as such, is essentially not bioavailable.

1.3.8 Flash point

Values of 208°C for polymeric MDI and 211°C for the 4,4’ isomer have been determined in the closed cup apparatus according to EC test method in compliance with GLP.

The IUCLID data sheet gives an additional number of values measured in open and closed cup but as information is not complete, they are considered as only supporting.

The lower validated value is used for risk assessment.

1.3.9 Autoflammability

The IUCLID data sheet quotes a value of “larger than 600°C” for autoflammability of both 4,4’ MDI and polymeric MDI when conducted to EC test methods and in compliance with GLP and this was accepted.

1.3.10 Explosivity

DSC/TGA analysis and their chemical structures suggest that neither polymeric MDI nor 4,4’ MDI are explosive.

1.3.11 Oxidising properties

Not an oxidising agent on the basis of the chemical structure.

1.3.12 Granulometry

A typical particle size distribution of 4,4'-MDI granules is 80% retained on 1.25 mm sieve with less than 1% fines.

1.3.13 Viscosity

The viscosity of MDI has been quoted as about 4.7 mPa s at 50°C for the 4,4' monomeric and 100 – 2,000 mPa s at 25°C for the polymeric substance (ICI, 1997).

1.3.14 Summary

The values in bold in the following table are judged as acceptable in that for each parameter the value has been determined by an acceptable method. As can be seen, in most cases the other data, which are less well reported, are consistent with the former.

Table 1.2 Summary of physico-chemical properties

Sect.	Property	Substance	Value	Method	R. i.*	Source
1.3.1	Physical state at ntp	4,4' MDI	White waxy solid	-	1	Annex VIIa
		PMDI	Dark amber liquid	-	1	Annex VIIa
1.3.2	Melting point	2,4' MDI	34-38°C	A1	1	Shell, 1994c
		4,4' MDI	39-43°C (39°C)	A1 (Capillary meth.)	1	Shell, 1994b
			40°C	A1 (DSC)	1	Shell, 1994b
			38°C	Not mentioned	4	Chadwick &Cleveland in: Kirk-Othmer 1981.
			38°C	Not mentioned	4	Dow, 1986a
			38°C	Not mentioned	4	Ryon, 1984 in MITES Report 0B001
			39°C	Not mentioned	4	III project AM-E-92
			37°C	Not mentioned	4	Occupational Health Guideline for MDI, 1978
			39.5°C	Not mentioned	4	Brochhagen & Schal, 1986
		PMDI	5°C	A1	1	Shell, 1994a
1.3.3	Boiling point (at 101325 Pa)	4,4' MDI	314°C	Not mentioned	4	Dow, 1986a
			314°C	Not mentioned	4	III project AM-E-92
			> 300°C	A2 (Siwoloboff)	1	Shell, 1994b
			364°C	A2 (DSC)	1	Shell, 1994b
			172°C	Not mentioned	4	Occupational Health Guideline for MDI, 1978
		PMDI	330°C	Not mentioned	4	Dow, 1986b
			> 300°C	A2 (Siwoloboff)	1	Shell, 1994a
			> 358°C	A2 (DSC/TGA)	1	Shell, 1994a
1.3.4	Relative density (at 20°C)	4,4/ MDI	1.325	A3	1	Shell, 1994b
		PMDI	1.238	A3	1	Shell, 1994a

Table 1.2 continued overleaf

Table 1.2 continued

Sect.	Property	Substance	Value	Method	R. i.*	Source
1.3.5	Vapour pressure (at 20°C)	2,4' MDI	0.0014 Pa	A4 (static)	1	Shell, 1994c
		4,4' MDI	0.0014 Pa	Not mentioned	4	III project AM-E-92
			0.019 Pa	Not mentioned	4	Ryon, 1984 in MITES Report 0B001
			6.7 Pa	Not mentioned	4	Occupational Health Guideline for MDI, 1978
			0.00049 Pa	See IUCLID	1	Brochhagen & Schal, 1986
			0.00074 Pa	Knudsen-effusion (weight loss)	1	Dow, 1989
			< 0.002 Pa	A4 (static)	1	Shell, 1994b
				Watson calculation from boiling point.		
		PMDI	< 0.005 Pa	A4 (static)	1	Shell, 1994a
				Watson calculation from boiling point.		
				Not mentioned		
			< 0.0013 Pa	Knudsen-effusion (weight loss)	4	Dow, 1986b
			0.00019-0.00077 Pa		2	Dow, 1989
1.3.6	Water solubility	Generic MDI	1.39 mg/l	See IUCLID	**	III Project FE-E-93
			0.378 mg/l		**	
			0.0201 mg/l		**	
			0.03 mg/l		**	
1.3.7	Partition coefficient (log Pow)	Generic MDI	4.5	HPLC (OECD n°117)	**	III Project FE-E-93
1.3.8	Flash point	4,4' MDI	211°C	A9	4	Annex VIIa
		PMDI	208°C	A9	4	Annex VIIa
1.3.9	Autoflammability	4,4' MDI	> 600°C	A15	4	Annex VIIa
		PMDI	> 600°C	A15	4	Annex VIIa
1.3.10	Explosive properties	DSC/TGA analysis and their chemical structures suggest that neither polymeric MDI nor 4,4' MDI are explosive				Annex VIIa

Table 1.2 continued overleaf

Table 1.2 continued

Sect.	Property	Substance	Value	Method	R. i.*	Source
1.3.11	Oxidising properties	None				Annex VIIa
1.3.12	Granulometry	4,4' MDI	A typical particle size distribution of 4,4' MDI granules is 80% retained on 1.25 mm sieve with less than 1% fines			Annex VIIa
1.3.13	Other data:					
	Viscosity	4,4'MDI: 4.7 mPa at 50°C PMDI: 100 – 2000 mPa at 25°C				ICI, 1997
	Surface tension	Not applicable since substance will react on contact with water				Annex VIIa
	Self ignition	None				Annex VIIa

* R. i. reliability index 1: method and description in accordance with test guidelines
2: falling short of highest standards concerning protocol or reporting
3: method or report not in accordance with test guidelines
4: minimal description of method and report

** see comments under Section 1.3.6 and 1.3.7.

1.4 CLASSIFICATION

1.4.1 Current classification

Environment

No classification or labelling for the environment.

Human health

Classification according to Annex I of the Directive 67/548/EEC (28th ATP, i.e. Dir. 2001/59/EC, O.J. 21.08.2001):

One entry (Index n° 615-005-00-9) in the Annex I applies to free diphenylmethane diisocyanate isomers:

Xn; R20 Xi; R36/37/38 R42/43

Labelling

R: 20-36/37/38-42/43
S: (1/2-)23-36/37-45

Explanation:

Xn	Harmful
Xn; R20	Harmful by inhalation
Xi; R36/37/38	Irritating to eyes, respiratory system and skin
R42/43	May cause sensitisation by inhalation and skin contact
S(1/2)	Keep locked up and out of the reach of children
S23	Do not breathe gas/fumes/vapour/spray (appropriate wording to be specified by the manufacturer; *p.m. to specify*)
S36/37	Wear suitable protective clothing and gloves
S45	In case of accident or if you feel unwell, seek medical advice immediately (show the label where possible)

Following concentration limits are applicable and the label must indicate the presence of the substance.

$C \geq 25\%$	Xn: R20-36/37/38-42/43
$5\% \leq C < 25\%$	Xn: R36/37/38-42/43
$1\% \leq C < 5\%$	Xn: R42/43
$0.1\% \leq C < 1\%$	Xn: R42

Nota 2 of Annex I (Dir. 67/548/EEC) mentions: The concentration of isocyanate stated is the percentage by weight of the free monomer calculated with reference to the total weight of the preparation.

Nota C: Some organic substances may be marketed either in a specific isomeric form or as a mixture of several isomers.

On the label must be stated whether the substance is a specific isomer or a mixture of isomers.

1.4.2 Proposal of the CMR WG

The proposal for revision of the classification of the substance recommended by the TC C & L WG for incorporation into the 30th ATP of Annex I is as follows:

Classification:	Carc. Cat. 3; R40// Xn; R20-48/20// Xi; R36/37/38 // R42/43	
Labelling:	symbol Xn R20-36/37/38-40-42/43-48/20 S(1/2-)23-36/37-45	 Nota C
Conc. Limits:		
C ≥ 25%	Xn; R20-36/37/38-40-42/43-48/20	
10% ≤ C < 25%	Xn; R-36/37/38-40-42/43-48/20	
5% ≤ C < 10%	Xn; R-36/37/38-40-42/43	
1% ≤ C < 5%	Xn; R40-42/43	
0.1% ≤C < 1%	Xn; R42	Nota 2

2 GENERAL INFORMATION ON EXPOSURE

2.1 PRODUCTION PROCESS

2.1.1 Production of MDI

Commercial synthesis of MDI takes place in closed systems and involves three major stages:

The production of MDA

Reaction of aniline with formaldehyde in the presence of HCl yields mixtures of 4,4'-MDA, 2,4'-MDA, 2,2'-MDA and condensation products which contain more than two aromatic groups.

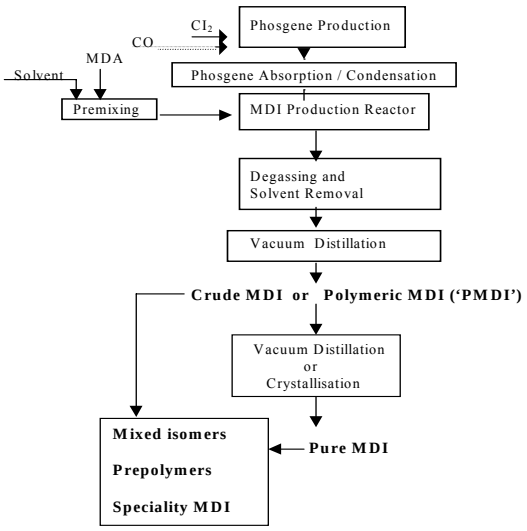
The phosgenation of these MDA molecules

Carried out at closely controlled temperature and pressure, results in a mixture of MDI di-isocyanate and polyisocyanate in solution together with traces of phosgene and HCl which are separated and recycled.

MDI purification

This stage involves separating the crude MDI, obtained after degassing and work up, to give polymeric MDI, pure MDI (4,4'-isomer) and mixed isomers. A variety of conditions may be employed and these will influence the proportions of the three materials isolated. Separation of the di-isocyanate mixture generally involves vacuum distillation at about 200°C using a steam ejector system and liquid ring pumps. Purification of MDI may also include a crystallisation stage.

Figure 2.1 Typical production route of MDI (source: Chief inspector's guidance to inspectors, HMSO 1990; adapted scheme)

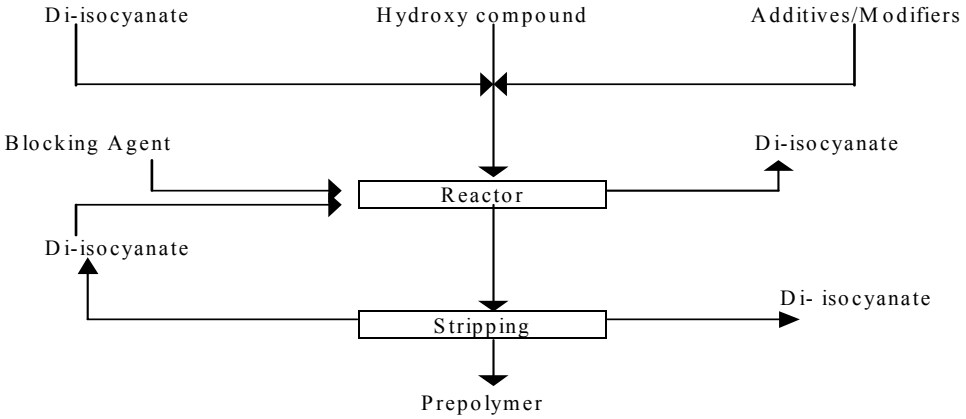


2.1.2 MDI prepolymer production

A typical production route for di-isocyanate prepolymers is given in **Figure 2.2**.

Figure 2. 2 Typical production route for di-isocyanate prepolymers (adapted from HMS), 1990)

Figure 2.1.1.: Typical production route for di-isocyanate prepolymers (adapted from HMSO, 1990)



Prepolymers of MDI are based on pure MDI, mixtures of MDI isomers or on polymeric MDI. Mixed prepolymer production with other di-isocyanates (for example TDI, isophorone-, p-phenylene- or hexamethylene di-isocyanates derivatives) is also possible.

The di-isocyanates are partly reacted with hydroxy compounds to give a mixture of compounds which are terminated in isocyanate or blocked isocyanate groups. These prepolymers are subsequently used in the manufacture of polyurethanes by further reaction with polyols.

A wide range of hydroxy compounds can be used in prepolymer production, including polyols and glycols. The polyols may be of the polyester or polyether type, analogous to components used in polyuethane foam manufacture. Special additives may be incorporated into the prepolymer reaction mixture in order to confer particular properties on subsequent formation of polyurethanes.

Di-isocyanate reaction

Di-isocyanate and hydroxy compound are pumped to reaction vessels and various additives may also be introduced into the mixture. The process is carried out in closed reactors with closely controlled heating and cooling. On completion of the required degree of reaction, a substance may be added which inhibits further reaction under the conditions employed. Blocking agents may also be introduced into the prepolymer mixture in order to form protecting groups for the isocyanate function which can be unblocked subsequently e.g. by application of heat prior to polyurethane manufacture. Further additions may be made and the product is then discharged into storage.

Modifying additives, inhibitors, blocking agents, etc may vary and the combination of such substances may be specific to the producer and/or application aimed at.

With mixed prepolymers based on TDI or aliphatic di-isocyanates, a stripping operation may be used to reduce monomeric di-isocyanate levels in the prepolymer. The TDI or other di-isocyanate is removed using a film evaporator and the material is recovered for re-use (Chief inspector's guidance to inspectors, HMSO 1990).

2.2 PRODUCTION CAPACITY

The world production, of MDI all types included, represents 1,2 million tonnes production in 1991 (SIDS). This production has increased to 2,5 million tonnes per year in 1996.

In Western Europe an increasing production trend is also seen: approximately 540,000 tonnes MDI were manufactured in 1993. In 1980, 267,000 tonnes were produced and 215,000 tonnes processed (Frey, 1990; CEH, 1994; cited in RAR MDA (EC, 2000)).

Figures on the EU production, import and export have been collected at 6 manufacturers sites falling actually under the Regulation (see **Table 2.1**). Sales volumes for 1996 were 790,000 tonnes (AnnexVIIA, 1998). Import into the EU lies in the range of 2,500-3,500 tonnes and the export out of the EU reaches 105,000 tonnes. Besides these six manufacturing companies (currently spread over 11 manufacturing sites in total), virtually no import of the substance takes place.

The amount produced in the EU is expected to continue to rise.

Table 2.1 Production sites of MDI in the EU (all > 1 000t/y; no company was located in the range 10 – 1 000 t/y, EUREX), IUCLID 1997 (ECB).

Company (and plant)	Country
Imperial Chemical Industries PLC (Hillhouse or Blackpool plant at Fleetwood)*	UK
ICI(1) Holland, Rotterdam	The Netherlands
Shell Nederland Chemie***	The Netherlands
Bayer Germany, Krefeld	Germany
Bayer Germany, Brunsbuttel	Germany
Bayer/Shell Belgium, Antwerp	Belgium
Bayer Hispania, Tarragona	Spain
Eni Chem(2) S.p.A. ; Brindisi	Italy
Dow Portugal Produtos Quimicos Lda. (Estarreja)	Portugal
Dow Deutschland Inc., Werk Stade	Germany
BASF Antwerpen N.V.	Belgium
BASF Schwarzheide GmbH	Germany
BASF AG Ludwigshafen**	Germany

* This plant was closed in 1997

** Since 1990 stepwise rundown of production and processing; since 1996 only research in pilotplants (amount < 1t): reason why this plant is considered actually as no more relevant f or the purpose of risk assessment

*** This plant is not a manufacturer site but takes products from Bayer/Shell venture in Antwerp

1) Huntsman purchased ICI Polyurethanes in 1999 (ISOPA, 2003)

2) Dow purchased Enichem in 2001 (ISOPA, 2003)

2.3 USE

The substance is mainly used in the industrial production of rigid polyurethane foams with world wide use. Many other uses are in the fields of coatings, adhesives, sealants and elastomers

(CASE) such as paints, adhesives, weather resistant sealing materials and footwear. There is use also in the production of particle board (bonding of wood) and mould cores for the foundry industry.

In rigid polyurethane production, low density products (30-50 kg/m^3) are mainly used for insulation purposes such as insulation panels, spray foam on walls and roofs, refrigerator insulation, oil storage tanks, refrigerated container transport and car accessories.

The life span of MDI and the corresponding tonnage are represented in the **Figure 2.3**.

Figure 2.3 The life-span stages and corresponding estimated volumes of MDI (on the basis of data from Industry data (1997) and B. Cope, pers. com., 1998)

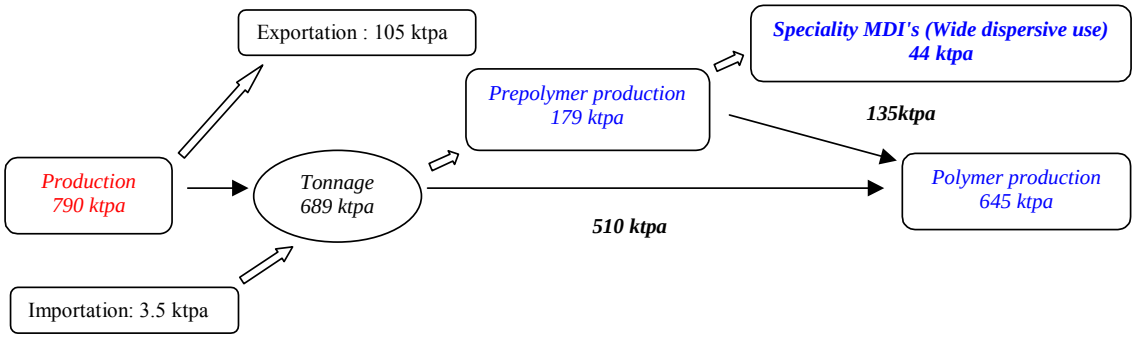


Table 2.2 Identified prepolymer production sites in the EU (non exhaustive list)

Company	Country
EniChem S.P.I [(1)], Cardano al Campo, Varese	Italy
BASF Germany, Schwarzheide	Germany
BASF Italy, Villanova d'Asti	Italy
BASF UK, Alfreton	UK
BASF Spain, Barcelona	Spain
BASF Germany, Lemforde	Germany
BASF Germany, Olchingen	Germany
ICI (2) Holland, Rotterdam	the Netherlands
COIM SpA, Settimo Milanese	Italy
Bayer Germany, Krefeld (?)	Germany

1) Dow purchased Enichem in 2001 (ISOPA, 2003)
2) Huntsman purchased ICI Polyurethanes in 1999 (ISOPA, 2003)

The proportion of MDI directly processed to prepolymers is 26% of tonnage (B. Cope, Polyurethane Consultant, pers. com.) that is 179 kt/annum – as well as a number of manufacturers plants, this processing takes place at distinct sites some of which collaborated in the data collection associated with this risk assessment (Industry via EU Questionnaire, 1997; B. Cope, personal com.; see **Table 2.2**). The proportion of MDI directly processed to polyurethane is 74% of tonnage i.e. 510 kt/annum.

It should be noted that some prepolymers will contain unreacted MDI, hence the processing of prepolymers was considered as a life cycle step of MDI. The proportion of unreacted MDI in prepolymers will vary according to their intended use. Since no data could be found concerning

the accurate proportion of MDI in these products, the total volumes of prepolymers produced were used when processing of MDI was considered.

Processing of prepolymers dedicated to applications of speciality MDI's (coatings, paintings, adhesives, sealants and cast elastomers) concerns about 6.5% of tonnage (B. Cope, pers. com) i.e. a volume of 44 kt/annum. This step takes place in numerous small companies and although MDI is not sold to the public some do-it-yourself products (e.g. one component foam, lacquers, varnishes, coating) may contain free MDI that would react immediately when coming in contact with air. To account for this the processing of these prepolymers was attributed to the main category IV (wide dispersive use) as a worst case assumption.

Categories considered in the risk assessment are as follow:

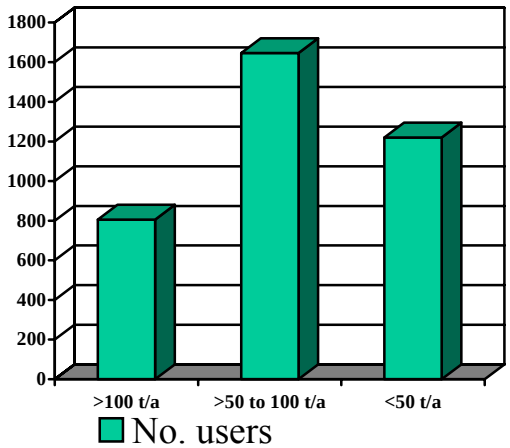
Table 2.3 Life span stages, categories and volumes used for estimation of MDI releases.

Life span stage	Industry category	Use category	Main category	Volume (ktpa)
Production	11 Polymers industry	43 Process regulators (dry monomers)	Ic Isolate intermediates with controlled transport	790
Processing to PU	11 Polymers industry	43 Process regulators (dry monomers)	III Non dispersive use	510
Processing to prepolymers	11 Polymers industry	43 Process regulators (dry monomers)	III Non dispersive use	179
Processing of prepolymers – speciality MDI's	11 Polymers industry	43 Process regulators (dry monomers)	IV Wide dispersive use	44
Processing of prepolymers other than speciality MDI's	11 Polymers industry	43 Process regulators (dry monomers)	III Non dispersive use	135

It concerns mainly industrial use, (predominantly) closed system or semi dispersive use or use resulting in inclusion into or onto matrix, and mainly continuous production. MDI is not sold to the public at large. However, some do-it-yourself-products may contain free MDI.

The users of the substance are numerous. An estimation of the number of users in relation to the volume used is given in **Figure 2.4**.

Figure 2.4 Estimation of Distribution of European MDI downstream Users (1996) (Source: ISOPA, 1999)



Remark: The estimate does not account for users of MDI in wide dispersive applications.

It is to be noted that release information provided by industry only concerns releases linked with the processing of 68 kt per annum; since the volume of MDI processed in the EU is of about 690 kt each year, the quantities released were extrapolated from these incomplete data. Industry is requested to provide further information concerning this point.

2.3.1 Use pattern and breakdown

A picture of the pattern of use and breakdown based on the data from an MDI producer survey and collated by III (Annex VIIA from ISOPA, 1997) is given in **Table 2.4**.

Table 2.4 Estimated percentage MDI usage in various applications

Rigid Polyurethane foam	Total	56%
Rigid foam laminates (continuous)		20
Rigid foam sandwich panels (discontinuous)		8
Appliances-refrigerators, freezers, boilers		16
Rigid block foam		3
Spray foam - roofing, boats		5
One component foam (OCF)		4
(Semi) Flexible Polyurethane Foams	Total	13%
Continuous flexible slabstock-furniture		2
Automotive-seating, trim, etc.		8
non load bearing structural parts		
Carpet backing		1
Office furniture		1
Packaging foam		1
C.A.S.E.	Total	26%
Coatings	Paints, lacquers	3
	Marine off-shore	<1
	Concrete flooring	<1
Adhesives	Binders for timber substitutes	9
	Athletic surfaces	<1
	Foundry moulds	3
	½ component adhesives	2
Sealants	Encapsulation of electrical joints	1
Elastomers	Shoe soles, print rollers	8
Thermoplastic Polyurethanes	Total	4%
Ski boots, cable sheathing, hoses		
Fibres	Total	1%

MDI is also used as grouting agent in rock consolidation or sealing of water leaks in tunnels or geotechnical construction works.

All MDIs are sold and used as manufactured; MDI is essentially used in an industrial environment although it cannot be excluded that some prepolymer preparation that still contain residues of MDI might be sold to the public in do-it-yourself products.

Usually, MDI's are not formulated by the addition of polyurethane processing additives such as catalysts, fire retardants, blowing agents etc.

These additives are formulated into the second component of the polyurethane system - the polyol.

2.3.1.1 Polyurethane Foams production

The production of low density polyurethane foams, for use as insulation material, uses a high proportion of the MDI produced in Europe.

In general the formulation to produce rigid polyurthane foam would consist of

1) A polyol	of low molecular weight
2) A catalyst	to control the reaction rate
3) A silicone oil	to control cell structure and stabilisation
4) A fire retardant	to impart fire resistance to the foam
5) A blowing agent	to expand the reacting mass to form a foam
6) MDI	to form polyurethane in reaction with the polyol

The ingredients 1 to 5 may be preblended or blended in line before the addition of the MDI.

The amount of MDI required in the formulation would be generally slightly in excess of the stoichometric amount required to react with the "active" hydrogen content of the other ingredients.

A rigid polyurethane foam has a uniform closed-cell structure. It finds many applications in the building industry, where its outstanding insulation properties and high strength to weight ratio make rigid polyurethane the preferred choice of material.

The formulation for a flexible polyurethane foam would be similar to the above however, it would employ a higher molecular weight polyol and a chemical known as a cell opener would be used in place of the silicone oil.

Flexible polyurethane foams are open-celled and are used mostly in furniture and bedding, where their unique properties provide a high comfort factor.

So called semi-flexible or microcellular foams are used in automotive applications.

Many of the production processes in this sector are continuous and lend themselves to automation, leading to a high safety factor.

Essential to the polyurethane processes are accurate metering of the components, efficient mixing of the chemicals and safe dispensing of the reacting mixture. Consequently, many of the processes and their ancillary equipment are totally enclosed.

Total MDI used: 475 kt/year

The chemistry used in polyurethane manufacture-Some basics:

1) the isocyanate group can react with any compound containing "active" hydrogen atoms;

2) polyurethanes are addition polymers formed by reaction of di- or poly-isocyanates with polyols;

3) the reaction of di-isocyanates with water yields a substituted urea and carbon dioxide. The initial product of the reaction is a substituted carbamic acid. Since carbamic acid is not stable it splits off carbon dioxide, forming the corresponding amine. The amine immediately reacts with further di-isocyanate in the reaction mixture forming urea;

4) secondary reactions of di-isocyanates may occur with urea and urethane linkages to form biuret and allophanate linkages respectively;

5) polymerisation ractions between di-isocyanates can lead to the formation of oligomers, uretidinediones (dimers), isocyanurate (trimers) or uretonimine .

2.3.1.2 C.A.S.E.

C.A.S.E. is an abbreviation for the group of applications that come under the headings

Coatings-Adhesives-Sealants-Elastomers.

Coatings and Paints

The most common two component paints consist of a "base paint" containing all of the constituents except the polyisocyanate. The latter is packed separately and mixed with the "base paint" just prior to use.

All commercial diisocyanates are of importance in the polyurethane coatings area.

MDI and its higher oligomers are used because of their relatively low vapour pressure at ambient temperature.

Industrial paints for the auto industry	25% of market
General industry and maintenance	25% of market
Wood and furniture	24% of market

(Data from Urethanes Technology)

Total MDI usage 26 kt/year.

Adhesives

Reactive adhesives consist of either two low molecular weight components - polyols and isocyanates. These react to form urethane in the adhesive mass or directly in the adhesive film or a liquid high molecular weight isocyanate terminated polyurethane. These react with ambient moisture to form the cured adhesive film.

Solvent based adhesives

These contain high molecular weight hydroxyl terminated polyurethanes and bond through the physical process of evaporation of the solvent.

Water based adhesives

- which contain a high molecular weight polyurethane dispersed in water. These bond through the physical process of drying or evaporation of water.
- which contain a water dispersible polyisocyanate and a high molecular weight polyurethane. These cure through chemical reaction as well as through the physical process of drying or evaporation.

Hot melt adhesives

- consist of high molecular weight hydroxyl terminated polyurethane in the form of a hot melt adhesive film. These bond through the physical process of cooling.
- consist of an isocyanate terminated polyurethane which is solid at ambient temperature but which melts at low temperature. These bond through the physical process of cooling as well as through chemical reaction with physical moisture.

MDI usage in C.A.S.E. 44 kt/year.

Binders

The process for making wood chips and fibre boards involves MDI compositions that are easily emulsified in water. Usually the MDI binders are processed without catalyst or other additives so that the need to prepare binder mixtures and the generation of liquid wastes is eliminated.

The low viscosity of the MDI emulsion allows for easy mixing with wood chips and low concentrations of binder may be used. The wood chip/binder mixture is pressed and heated to form the cured final product, a particle/fibre board.

MDI usage 60 kt/year.

Foundry moulds

Foundry moulds, for the production of cast iron or aluminium products, are produced from sand and an MDI binder.

In the Ashland process, a phenolic resin and polymeric MDI are mixed with sand. The mixture is poured into a mould in an enclosed box. A rapid curing reaction is initiated by passing a stream of volatile amine catalyst (carried by air or carbon dioxide) through the mould.

The approximate ratio of binder to sand is 1 to 2%.

MDI usage 20 kt/year.

Sealants

One component polyurethane systems cure by contact of the surface with atmospheric moisture. The isocyanate groups of low NCO content prepolymers of MDI and polyether polyols have sufficient reactivity towards water so that the reaction proceeds in a controlled manner, liberating carbon dioxide gas and forming urea linkages.

Major applications:

- Sports surfaces - all weather running tracks

- Jointing compounds - cars (windscreen), ship building, containers and aircraft.

MDI usage 3 kt/year.

Elastomers

Cast elastomers are produced by metering the two components, simultaneously mixing and pouring the reacting mixture into a mould.

The diisocyanate component may be a modified pure MDI or a prepolymer, where a portion of the NCO groups have been reacted with a polyol.

Cast elastomers - wheels, tyres, rollers, and mining.

Casting resins - electrical joints, encapsulation.

MDI usage 5 kt/year.

Shoe soles

Shoe soles may be manufactured by moulding directly on to the shoe upper or as separate shoe soles which may be bonded to the shoe uppers by an adhesive at a later stage.

The polyurethane sole may be formed from a modified pure MDI in reaction with a chain extender (e.g. 1,4 butanediol).

The modified MDI can have a % NCO content of approximately 20% but a zero to low MDI monomer content.

MDI usage 55 kt/year.

2.3.1.3 Thermoplastic Polyurethanes

Thermoplastic polyurethanes (TPUs) are usually made from pure MDI which is reacted with a substantially linear polyether or polyester diol and with a chain extending diol of low molecular weight (1.4 butanediol) in either a one step or two step process.

Most grades of TPU can be injection moulded to produce moulded gears and bearings used in engineering applications.

Profiles, tubes, hoses and cable sheathings find use in electrical applications.
One of the largest single applications is the production of moulded sports shoes and ski boots.

MDI usage 25 kt/year.

2.3.1.4 Polyurethane Fibres (Spandex)

The polyurethane component is usually made by a continuous two stage process using a di-isocyanate terminated prepolymer that is chain extended with a low molecular weight diol or diamine.

Fibres may be spun by dry spinning from solution, wet spinning, reaction spinning and hot melt extrusion. Probably the most important process is dry spinning from a dimethyl formamide solution in which polymerisation takes place.

The fibres are used in clothing, replacing natural rubber threads in some applications such as surgical stockings, swim wear and stretch fabrics for clothing and furnishings.

MDI usage 5 kt/year.

3 ENVIRONMENT

3.1 ENVIRONMENTAL EXPOSURE

3.1.1 Environmental releases

Synthesis of MDI takes place in closed systems where the reaction of aniline with formaldehyde in the presence of HCl yields mixtures of 4,4'-MDA (methylenedianiline), 2,4'-MDA, 2,2'-MDA and condensation products which contain more than two aromatic groups. The phosgenation of these MDA molecules produce MDI. The product obtained is a mixture of 4,4'-MDI, 2,4'-MDI, 2,2'-MDI and polymeric MDI which consists of homologues of monomeric MDI. Pure 4,4'-MDI isomer can be obtained by distillation of the reaction products.

Since the NCO groups of MDI react readily with OH groups, contact of MDI with water is carefully avoided through production and storage stages. Consequently releases of MDI to effluents are expected to be virtually zero at the production sites. Likewise, releases to soil and sediment are also expected to be negligible. Releases of MDI to atmosphere might occur during production although they are expected to be low; moreover exhaust gazes produced during the MDI formation reaction are treated by incineration or scrubbing of MDI vapours.

The produced MDI is transported in road tankers, tank containers, rail tank cars or drums either from the production site or is conveyed in pipelines to loading or using sites. Road tankers and tanks are pressurised with dry air or nitrogen to eliminate contact of MDI with moisture of ambient air. Cleaning of road tankers and tank containers with water is rarely performed. Drums which have contained MDI are decontaminated with, for example, polyethylene glycol and tenside aqueous solutions. Waste water resulting from cleaning of road tankers and tank containers and decontamination procedures is not considered to contain any MDI but rather polyurethanes and polyureas which are insoluble and inert compounds.

The MDI produced is mainly directly processed for the synthesis of polyurethanes. MDI is mixed with OH containing products such as polyols. The di-isocyanates are completely reacted after a few hours although thorough curing might be further achieved by incubations at elevated temperatures. Emissions of MDI to soil, waste water and thus sediment linked to this processing stage are expected to be negligible and polyurethane products neither contain generic isocyanates nor biologically available isocyanate groups (Dieterich et al., 1994). Since the reaction of MDI and OH groups is exothermic, it is possible that some MDI might evaporate near the exit of the device where MDI and polyols are mixed (mixing head) nevertheless the proportion of MDI which indeed reaches the atmosphere is likely to be low because of the low vapour pressure of MDI and the further treatment of the vapours collected at this stage.

Part of the MDI produced is processed in prepolymers by adding di- or polyhydroxy compounds to MDI with a molar excess of the di-isocyanate. Prepolymers are mainly used for production of cast elastomers; they are also used in the production of elastomeric fibres, one-component coating systems and most PU (polyurethane) based sealants.

Main applications for MDI are the synthesis of rigid and (semi-) flexible foams (69%) and coatings, adhesives, sealants and elastomers (C.A.S.E., 26%). For a detailed listing of the applications see Section 2.3.1. A somewhat particular use of MDI is its utilisation as grouting agent in tunnels; a laboratory scaled study conducted in the frame of the Romeriksporten tunnel construction (Norway) where MDI has been used to reduce water leaks from the tunnel indicated

that the levels of MDI and MDA released from the foam to surrounding water were negligible (SFT, Norwegian Pollution Control Authority, pers. com., Nov. 1999). Although MDI and MDI prepolymers are mainly processed in big plants some applications of MDI concern small companies or may even be sold to the public at large in do-it-yourself products. So releases relevant for processing of these latter types of applications (which essentially concern C.A.S.E. products) may not be well controlled or defined. This aspect was tackled by allocating C.A.S.E. prepolymer processing to the main category IV (wide dispersive use) for performing the risk assessment as worst case approach.

3.1.2 Degradation

3.1.2.1 Water

Hydrolysis

When MDI is added to water its NCO groups react readily with OH groups of the water to form a mixture of di-isocyanates and amines which then readily react with more MDI to produce inert, insoluble solid polyureas. Under conditions typical of many types of environmental contact, i.e. with relatively poor dispersion of the heavy di-isocyanate, the interfacial reaction leads to the formation of a solid crust encasing partially unreacted or unreacted material. This crust restricts ingress of water and egress of amine, and hence slows and modifies hydrolysis.

The impact of the formation of this urea crust can be seen in studies addressing the impact of stirring on the hydrolysis of MDI. A study conducted by Yakabe et al. (1992) to determine the fate of polymeric MDI (PMDI) in water established that the disappearance rate of MDI was higher when it was dispersed in the medium through high speed stirring (2.5%/hour at 25°C) rather than through moderate shaking (1.5%/hour at 25°C). The disappearance rate of MDI reduced to approximately one fourth when the reaction temperature was lowered from 25 to 12°C. Yakabe et al. (1999) showed that the disappearance rate of MDI was similar with loadings from 400 mg L^{-1} to 10,000 mg L^{-1}; they also report that the use of synthetic seawater as experimental media did not appreciably alter the reaction kinetics. So according to these data the degradation rate of MDI spilled in a natural aquatic environment that is in media where hydrological conditions (turbidity, discharge) correspond to a moderate mixing of the spilled product and at temperatures possibly as low as 12°C, the half-life of MDI would be of about 143 hours i.e. a rate constant for hydrolysis of 0.116332/day. Concentration of MDI did not significantly affect disappearance rates between 400 to 10,000 mg/l.

MDI is inherently reactive in water, with a predicted half-life in solution of a minute or less (Yakabe et al., 1999). Because of this high reactivity, MDI can have only a transient existence in water, and as such, is essentially unavailable for uptake and bioaccumulation or for biodegradation. Heimbach (1993) detected no MDI (detection limit: 0.006 mg/l) in artificial 4.5 m^3 ponds when polymeric MDI had been added up to a concentration of 10 g/l from day 0 to day 112 of experimentation. The methods used employed derivatisation with dibutylamine followed by HPLC analysis and derivatisation with N-(4-nitrobenzyl) propylamine followed by TLC analysis.

Likewise, MDI was not detected in any study reported, where the MDI concentration in the experimental aqueous media after addition of MDI was determined.

Methylenedianiline (MDA) concentrations yielded by the reaction of MDI with water are always low and especially with low shear dispersion of MDI in the water which corresponds to the likely conditions under which MDI spilled in natural waters would be dispersed. For example Yakabe et al. (1992) measured an average concentration of 5 mg of MDA per litre of medium after 4 to 50 hours when 1,000 mg of polymeric MDI was added to 1 litre water (magnetic stirring at 3,000 rpm) which coresponds to a MDA yield of 0.128%. Under static conditions, Yakabe et al. (1999) measured an MDA yield of 0.005%. Heimbach (1993) who poured PMDI (up to 10 g/l) in artificial ponds under relatively static conditions which relate closely to environmental spills detected no MDA in water (detection limit: 0.014 mg/l). In the Rhône-Poulenc report (1977) an initial concentration of 500 mg/l of MDI added to the fish medium by stirring for 18 hours yielded concentrations of respectively 3.5 mg/l and 4 mg/l of MDA for polymeric MDI and 4,4'-MDI after a 24 hours incubation which correspond to yields of 0.7 and 0.8%. Addition of 100 mg of MDI to one litre of distilled water yielded a MDA concentration of 2 mg/l i.e. 2% of initial MDI concentration. Fujiwara (1981) reports a MDA yield of 0.54% 24 hours after addition of 50 ppm polymeric MDI to a river model device.

So yields of MDA when MDI is added to aqueous media are comprised between 0.128 and 2%.

Biodegradation

Caspers et al. (1986) performed the Activated sludge, respiration inhibition test (OECD 209) and the Inherent biodegradability: modified MITI test (II) (OECD 302C) with polymeric MDI (49.7% of monomer). They found no inhibition of activation sludge respiration with up to 100 mg of MDI/l of experimental medium. They also failed to detect any biodegradation in the MITI test with a concentration of 30 mg/l even after 28 days. It is to be noted that these results probably do not concern MDI but rather its reaction products with water i.e. mainly polyureas since MDI reacts readily with water and the tests mentioned here above were performed in aqueous media.

The inherent biodegradability test (OECD 302 C) was also carried out by Yakabe (1995) with oligoureas yielded by the reaction of 4,4'-MDI and 4,4'-diaminodiphenylmethane. The average percent biodegradability of these compounds estimated from BOD and residual test substance were respectively of 0% and 6.4%.

It can be concluded from these studies that MDI and its reaction products with water are not degraded by micro-organisms.

Photolysis

There are no data concerning photolysis of MDI in water but considering the high rate of hydrolysis it can be assumed that photolysis is not a relevant process in the MDI degradation.

3.1.2.2 Soil

No degradation experiment was performed with MDI in soil. It can nevertheless be assumed that MDI isomers and polymers will probably readily react with soil moisture to form polyureas.

Since data on the degradation rate of MDI in soil are not available, the rate constant for degradation provided by the EUSES program ($6.93 \cdot 10^{-7}$/day) was used to perform the risk assessment in this compartment. The value of this parameter is probably lower than the real one but it was used as a worst case value in the absence of any other data.

3.1.2.3 Sediment

In the study conducted by Heimbach (1993), measurements of MDI were performed in the sediment. Concentrations decreased throughout the study from 7.6 to ≤ 0.7 mg/kg in the low dosed pond (1 g/l) and from 20 to 0.8 mg/kg in the high dosed pond (10 g/l). Degradation rate was not constant throughout the study. Half-lives of MDI in the urea residues found in sediment varied respectively from 7 to 80 days and from 14 to 28 days in these ponds. A half-life of 80 days was considered for calculating rate constant degradation of MDI in sediment.

3.1.2.4 Atmosphere

The atmospheric reaction rate of MDI with hydroxyl radical was calculated by Bailey (1993). He used the computer program of Meyland and Howard which is based on the formalism of Atkinson (Atkinson, 1988). The tropospheric half-life of 4,4'-MDI was of 1.331 days at tropospheric concentration of 5.10^5 OH molecule/cm^3. This same computer program was also used to calculate the tropospheric half-life of TDI; the result yielded (2.426 days) is similar to that calculated by Becker et al. (1988) on the basis of observations of reactions of TDI with OH radicals in a photoreactor filled with synthetic air (2.167 days).

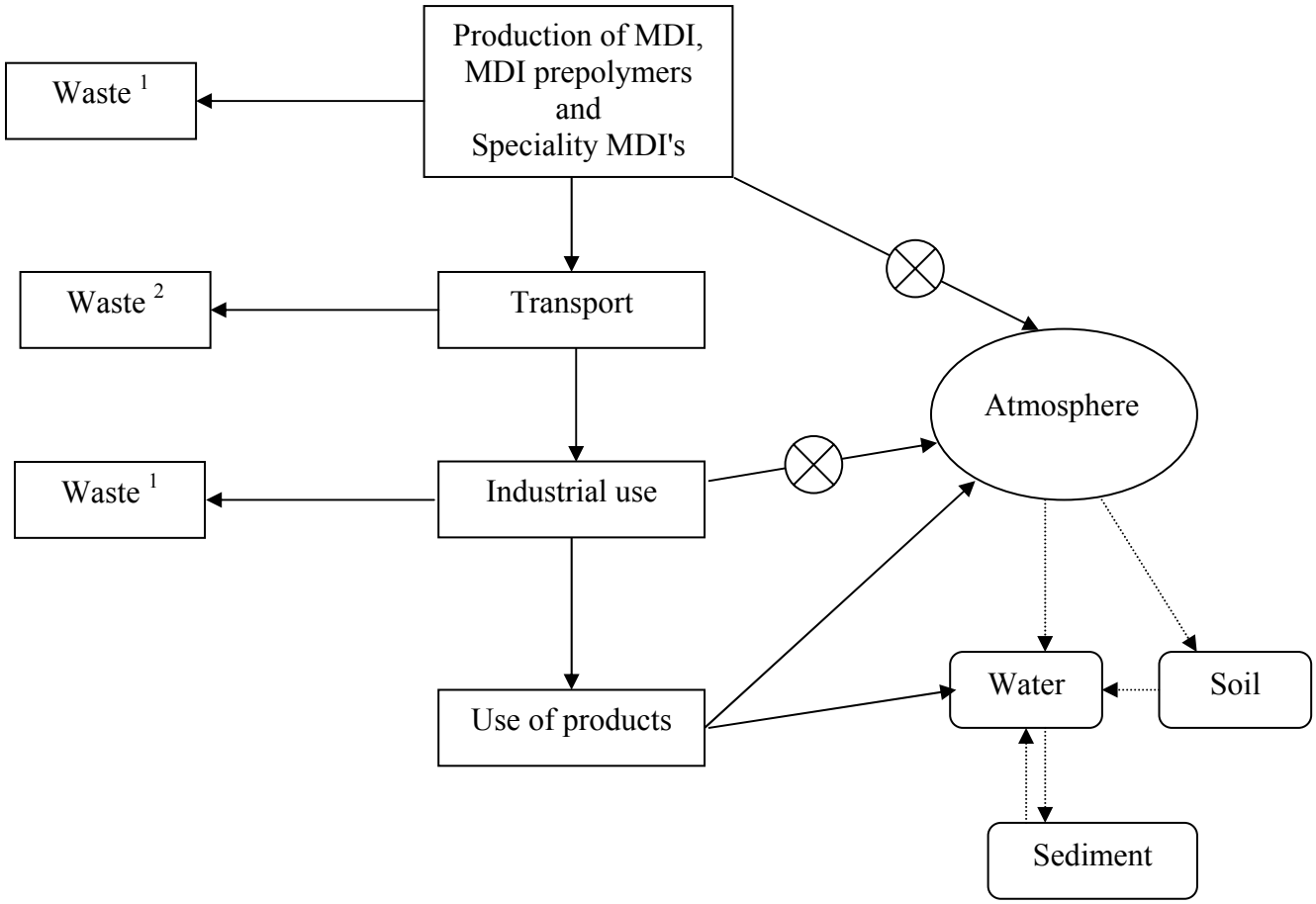
Degradations rates used for the risk assessment are as follows:

Rate constant for biodegradation in STP:	0 (d^{-1})
Rate constant for degradation in air:	0.521 (d^{-1})*
Rate constant for hydrolysis in surface water:	0.116332 (d^{-1})**
Rate constant for biodegradation in surface water:	0 (d^{-1})
Rate constant for degradation in bulk soil:	$6.93.10^{-7}$ (d^{-1})***
Rate constant for degradation in bulk sediment:	$8.66.10^{-3}$ (d^{-1})****

*	based on calculations of Bailey (1993)
**	based on the disappearance rates reported by Yakabe et al.(1992) with moderate shaking at 12°C (0.35%/hour).
***	default values provided by EUSES program.
****	based on the data reported by Heimbach (1993)

Potential releases and fate of MDI in the environment are summarised in **Figure 3.1**.

Figure 3.1 Potential releases and fate of MDI in the environment.



Waste 1 Wastes from production and processing sites of MDI and prepolymer are incinerated by specialist contractors.

Waste 2 Tanks dedicated for the transportation of MDI are cleaned out after several years of service (2-5 years) by hydroblasting. Specialist contractors carry out the service and the effluent is generally incinerated.

⊗ Exhaust gases possibly treated by incineration in special combustion chambers, scrubbing off or filtration through activated carbon filters.

⋯▸ Transport considered negligible considering the short half-life of MDI in the atmosphere, water and moistened soil

3.1.3 Distribution

The distribution of MDI in the different environmental compartments yielded by multimedia models should be considered with great caution; indeed the very high reactivity of MDI with water is not accounted for by these models.

Nevertheless it can be stated that the major releases of MDI will be to the atmosphere. When considering the short half-life of MDI in this compartment it is unlikely that significant amounts of the emitted substance will undergo deposition and further contaminate soil or water and sediment.

3.1.4 Accumulation

Two studies were found where the potential accumulation of MDI in aquatic systems was tested. In the study by Heimbach et al. (1996) up to 10 g of polymeric MDI was added per litre of water into artificial outdoor ponds and the concentration of MDI was monitored for 112 days in all compartments (water, fish (*Oncorhynchus mykiss*) and sediment). The authors never detected any traces of MDI nor MDA (methylenedianiline) in water and fish throughout the whole experimentation period; the detection limit of MDI and MDA in water was of 5-10 µg/l and it was respectively of 1.45 mg/l and 0.5 mg/kg for MDA and MDI monomer in fish. In another study by Fujiwara (1981) *Cyprinus carpio* (fish) did not accumulate any MDI (detection limit 0.1 mg/kg) after 8 weeks in a river model to which was added 0.1 mg/l of polymeric MDI.

These studies give evidence that MDI accumulation through aquatic food chains is very unlikely to happen.

Table 3.1 Life span stages, categories, volumes and tables used for estimation of MDI releases.

Life span stage	Industry category	Use category	Main category	Volume (ktpa)	A-table	B-table
Production	11 Polymers industry	43 Process regulators (dry monomers)	Ic Isolate intermediates with controlled transport	790	A 1.1	B 1.14
Processing to PU	11 Polymers industry	43 Process regulators (dry monomers)	III Non dispersive use	510	A 3.10 Type I, dry	B 3.9
Processing to prepolymers	11 Polymers industry	43 Process regulators (dry monomers)	III Non dispersive use	179	A 3.10 Type I, dry	B 3.9
Processing of prepolymers – speciality MDI's	11 Polymers industry	43 Process regulators (dry monomers)	IV Wide dispersive use	44	A 3.11 Type V	B 3.9
Processing of prepolymers other than speciality MDI's	11 Polymers industry	43 Process regulators (dry monomers)	III Non dispersive use	135	A 3.10 Type I, dry	B 3.9

3.1.5 Predicted environmental concentrations (PECs)

The volume of MDI produced in EU is 790 kilotons of which 105 kt are exported out of EU. Only 3.5 kt were imported, so tonnage is 689 kt. These figures are those collected by ISOPA (European Diisocyanate and Polyol Producers Association) 1997 and Industry (1997) for the reference year 1996.

The life span stages considered for exposure assessment are listed in **Table 3.1** together with the classification in adequate categories, the volume of chemical concerned and the A and B tables of TDG used to estimate releases.

As can be seen five separate stages were considered:

- production,
- processing of MDI to polyurethane foams, thermoplastics, fibres and C.A.S.E. applications not based on prepolymers; (this category is designed in **Table 3.1** by processing to PU),
- processing of MDI to prepolymers
- processing of prepolymers – "speciality MDI's"
- processing of prepolymers other than "speciality MDI's".

The proportion of MDI directly processed to prepolymers is 26% of tonnage (B. Cope, Polyurethane Consultant, pers.com.) that is 179 kt/annum. Consequently the proportion of MDI directly processed to polyurethane is 74% of tonnage i.e. 510 kt/annum.

It should be noted that prepolymers will contain some unreacted MDI, hence the processing of prepolymers was considered as a life span step of MDI. The proportion of unreacted MDI in prepolymers will vary according to their intended use. Since no data could be found concerning the accurate proportion of MDI in these products the total volume of prepolymers produced were used when processing of MDI still present in prepolymer mixtures was considered.

Processing of prepolymers dedicated to applications of speciality MDI's (coatings, paintings, adhesives, sealants and cast elastomers) concerns about 6.5% of tonnage (B. Cope, pers. com.) i.e. a volume of 44 kt/annum. This step takes place in numerous small companies and it is possible that some MDI containing products might be sold to the public at large as do-it-yourself product. To account for this the processing of these prepolymers was attributed to the main category IV (wide dispersive use) as a worst case assumption.

PECs were calculated on the basis of emission factors as included in release table of TGD and specific release information provided by industry. Indeed, information on measured or estimated emissions was provided for numerous sites. The information concerned releases to air, water and amounts of wastes with the procedure followed for their disposal. Release data provided by industry are gathered in **Table 3.2**.

Table 3.2 Releases information provided by producers and users.

Production sites	Volume produced (ktons/year)	Emission to air (kg/year)	Emission to water (kg/year)	Wastes (tons/year)
Site 1	90	3	0	200 (incinerated)
Site 2	54	Not currently measured ⇨ *6.5**	0	< 5 (incinerated or in authorised landfill)
Site 3	120	0[1]	No emission to hydrosphere	No data
Site 4	44	0[1]	No emission to hydrosphere	No data
Site 5	36	0[1]	No emission to hydrosphere	No data
Site 6	76	0[1]	No emission to hydrosphere	No data
Site 7	70	- ⇨ 8.4*	-	20 (incinerated)
Site 8	67	0	0	2 (incinerated)
Site 9	36.3	0.17	Not applicable	-
Site 10	30.3	0.0553	0	3 (incinerated)
Site 11	166	20	0	20.3 (incinerated)
Total for production	790	38	0	5[£]
PROCESSING To Prepolymer	Volume processed (kt/year)	Emission to air (kg/kt MDI processed)	Emission to water (kg/year)	Wastes (tons/year)
Site 12	3.30	0.121	Not applicable	-
Site 13	3.00	3.833	0	0
Site 14	1.71	5.497	0	3 (incinerated)
Site 15	1.50	5.000	0	0
Site 16	13.60	4.118	0	0
Site 17	5.80	5.000	0	0
Site 18	11.00	5.036	0	0
Site 19	15.00	0.333		
Maximal emission ratio (kg/kt MDI processed)	-	5.497	0	0
Processing to PU	Volume processed (kt/year)	Emission to air (kg/kt MDI processed)	Emission to water (kg/year)	Wastes (tons/year)
Site 20 (1995)	0.50	12	0	0
Site 20 (1998)	0.50	<0.5		
Site 21	3.50	0[1]	0	0
Site 22	1.00	1	0	0
Site 23	3	0.025	0	0

Table 3.2 continued overleaf

Table 3.2 continued Releases information provided by producers and users.

Processing to PU	Volume processed (kt/year)	Emission to air (kg/kt MDI processed)	Emission to water (kg/year)	Wastes (tons/year)
Site 24	0.77	0.8	0	0
Site 25	0.32	3	0	0
Site 26	0.33	11	0	0
Site 27	2.60	0.2	0	0
Site 28	3.00	1	0	0
Site 29	0.12	0.2	0	0
Site 30	0.14	6	0	0
Site 31	0.12	0.1	0	0
Site 32	6.8	0.001		
Site 33	3.5	0.006		
Site 34	6	0.003		
Site 35	1.2	<0.001		
Site 36	0.5	<0.001		
Maximal emission ratio: (kg/kt MDI processed)	-	12	0	0

1) Exhaust air burned off by special combustion chambers.
* Calculated values
£ Most of the wastes' volume mentioned by industry are incinerated, and thus not relevant for environmental risk assessment. However, for production site 2, '<5 t/year' are incinerated or disposed on landfill. As a worst case, it was considered that all of this volume was disposed on landfill and the figure '5' was used to perform the environmental risk assessment.

The amounts released to air presented in **Table 3.2** are either calculated on the basis of intermittent or programmed measurements of MDI emissions and rate of exhaust emission or are estimated from saturation concentration and movement of tanks. For two production sites out of 11 (sites 2 and 7), emissions to air were not provided. For these sites the emissions were estimated as follow: the emissions ratios were calculated for production sites for which emission data (amount of MDI emitted to air per year/ amount of MDI produced per year) were available. The emissions for site 2 and 7 (values marked with an asterisk in **Table 3.2**) were estimated by multiplying the highest emission ratio to air from the ratios calculated for all the other sites, by the amount of MDI produced at sites 2 and 7.

On the basis of these data and extrapolations the total continental releases to air due to production were calculated by summing all releases to air due to production i.e. 38 kg/annum as shown in **Table 3.2**; regional releases are thus estimated to be of 3,8 kg/annum (one tenth of continental releases).

Moreover, emission to air for processing were not available for all sites; the total amount of MDI processed in the sites for which data was provided is of 88 kt/annum when the total volume of MDI processed in the EU was 689 kt/annum. Emissions for total processing were extrapolated on the basis of the highest emission ratio calculated for processing to prepolymers and processing to polyurethanes respectively (see **Table 3.2**). Continental release to air due to processing to prepolymers is thus 985 kg per annum (179 ktpa processed and a release ratio to air of 5.497 kg per kt processed at worst, as diplayed in **Table 3.2**); regional releases are 98.5 kgpa

(one tenth of continental emissions). Continental release to air due to processing to polyurethanes is thus 6,120 kg per annum (510 ktpa processed and a release ratio to air of 12 kg per kt processed at worst as displayed in **Table 3.2**); regional releases are 612 kgpa (one tenth of continental emissions).

Amounts of MDI released to water are considered to be zero; indeed, whenever measurements in effluents were carried out, no MDI could be detected in water as expected on the basis of the hydrolytic behaviour of MDI.

Amounts of MDI released to industrial soil were overridden in the model for production; indeed <5 000 kgpa are reported to be disposed in authorised landfills so it was considered that continental releases to this compartment were 5,000 kg/annum.

Finally, the fraction of the main local source for production could be calculated as follows:
166 kt/annum (maximum production at one site)/790 kt/annum (total production in the EU) = 0,21; this value was inputted into EUSES.

A summary of overriden values for release estimates and fractions based on release information provided by industry are presented in **Table 3.3**.

Table 3.3 Release estimates and fractions based on release information provided by industry that have been overriden to values outputed by EUSES.

Life cycle stage	Data overridden for the continental environment	Data overridden for the regional environment	Data overridden for the local environment
Production	release to air: 38 kg y^{-1}[(a)] release to industrial soil: 5,000 kg y^{-1}[(a)]	release to air: 3.8 kg y^{-1}[(b)] release to industrial soil: 500 kg y^{-1}[(b)]	fraction of main local source: 166 kty^{-1}[(c)] = 0.21 790 kty^{-1}[(d)]
Processing to PU	release to air: 510 kg y^{-1}[(e)] · 12[(f)] = 6,120 kg y^{-1}	release to air: 51 kg y^{-1}[(b)] · 12[(f)] = 612 kg y^{-1}	
Processing g to prepolymers	release to air: 179 kg y^{-1}[(g)] · 5.497[(h)] = 985 kg y^{-1}	release to air: 17.9 kg y^{-1}[(b)] · 5.497[(h)] = 98 kg y^{-1}	

a) Sum of release information provided by industry (see Table 3.2)
b) Release information provided at the continental divided by 10 for transposition to the regional environment (see TGD)
c) Maximal volume produced at one site as shown in Table 3.2
d) Total volume produced as shown in Table 3.2
e) Estimated total MDI volume processed to PU according to information provided by ISOPA in 1996 (see text)
f) Maximal emission ratio for processing to PU (see Table 3.2)
g) Estimated total MDI volume processed to prepolymers according to information provided by ISOPA in 1996 (see text)
h) Maximal emission ratio for processing to prepolymers (see Table 3.2)

PECs calculated with the EUSES program are presented in **Table 3.4** and **Table 3.5**. Input data and detailed results of the program calculations are presented in a separate file annexed to the report.

Table 3.4 Local PECs

Environmental compartment	Production	Processing to PU	Processing to prepolymers	Processing of prepolymers speciality MDI's	Processing of prepolymers other than speciality MDI's
Air (mg/m^3)	$8.14\ 10^{-7}$	$2.35\ 10^{-5}$	$7.71\ 10^{-6}$	$5.25\ 10^{-6}$	$1.\ 05\ 10^{-5}$
Surface water (mg/l)	$1.37\ 10^{-6}$	$1.37\ 10^{-6}$	$1.37\ 10^{-6}$	$1.37\ 10^{-6}$	$1.37\ 10^{-6}$
Agricultural soil (mg/kg WWT)	$5.38\ 10^{-5}$	$4.85\ 10^{-4}$	$1.85\ 10^{-4}$	$1.38\ 10^{-4}$	$2.38\ 10^{-4}$
Grassland (mg/WWT)	$6.00\ 10^{-5}$	$7.22\ 10^{-4}$	$2.61\ 10^{-4}$	$1.89\ 10^{-4}$	$3.43\ 10^{-4}$
Sediment (mg/WWT)	$1.66\ 10^{-4}$	$1.66\ 10^{-4}$	$1.66\ 10^{-4}$	$1.66\ 10^{-4}$	$1.66\ 10^{-4}$

Table 3.5 Regional PECs

Environmental compartment	Regional PEC
Surface water	$1.38\ 10^{-6}$ mg/l
Air	$2.06\ 10^{-7}$ mg/m^3
Agricultural soil	$4.21\ 10^{-5}$ mg/kg WWT
Natural soil	$4.23\ 10^{-5}$ mg/kg WWT
Sediment	$6.94\ 10^{-5}$ mg/kg WWT

3.2 EFFECTS ASSESSMENT: HAZARD IDENTIFICATION AND DOSE (CONCENTRATION)-RESPONSE (EFFECT) ASSESSMENT

All data reported hereafter concern polymeric MDI, monomeric MDI or even mixtures of both. However, it is very likely that the toxicity of generic MDI to species involved in ecotoxicity tests is similar to that of the tested MDI mixtures. In all tests performed, MDI had to be either added to water or to some moistened material. Considering the high reactivity of MDI with water it is most probable that it was the breakdown products that were being tested not MDI. Indeed, when attempts were made to measure MDI concentrations in experimental media, the results were always below the detection limits as in Heimbach's (1993) study where water samples removed from two ponds, dosed with 1g/l and 10g/l polymeric MDI, on days 0, 1, 7, 14, 28, 56 and 112 and were found to contain levels of MDI lower than the detection limit (i.e. 5-10μg l^{-1}).

The hydrolysis products of MDI and water are dependent on the conditions of the mixing of the MDI with water. Under conditions of low dispersion the immediate products are insoluble, solid and inert polyureas. Under conditions of high dispersion, some MDA (methylenedianiline) as an initial hydrolysis product may be formed, however, given its extreme reactivity with MDI, MDA is rapidly transformed. Thus when measurements were made, concentrations ranged from 5 mg/l to lower than the detection limits. Nevertheless, since MDA is very toxic to organisms (see MDA Risk Assessment Report (EC, 2000) and EC_{50} values reported in Section 3.2.1 and 3.2.3), the possible hazard to aquatic organisms due to MDA formation when MDI enters the water compartment is not to be overlooked. This aspect is treated theoretically in Section 3.3.

Effect data are presented here after; the studies reported are scored as follows:

- a reliability index of 1 was assigned to studies for which the methods and reporting are in accordance with test guidelines and that include accurate concentration measurements;
- a reliability index of 2 was assigned to studies for which the methods and reporting are in accordance with test guidelines but that do not include accurate concentration measurements or fall short of highest standards concerning protocol or reporting;
- a reliability index of 3 was assigned to studies for which method or reporting were not in accordance with test guidelines;
- a reliability index of 4 was assigned to studies where description of method and results is minimal.

As the data set on effects is rather limited, all data are shown regardless of their reliability index.

3.2.1 Aquatic compartment

3.2.1.1 Fish

The MDI toxicity studies with fish are presented in **Table 3.6** and **Table 3.7**.

Table 3.6 MDI toxicity to fish: short-term studies.

Short-term studies							
Substance tested	Concentrations tested (mg/l)	Species	Method	Endpoint (mg/l)	MDA analyses	Reliability index	Reference
Polymeric MDI	500	*Brachydanio rerio*	Other, 24 hours, static, no analytical monitoring, MDI dispersed by magnetical stirring for 18 hours.	$LC_0 \geq 500$ $EC_0 \geq 500$ (behaviour)	3.5 mg/l after 24 hours	3	(Rhône-Poulenc, 1977)
4,4' MDI	500	*Brachydanio rerio*	Other, 24 hours, static, no analytical monitoring, MDI dispersed by magnetical stirring for 18 hours.	$LC_0 \geq 500$ $EC_0 \geq 500$ (behaviour)	4 mg/l after 24 hours	3	(Rhône-Poulenc, 1977)
Polymeric MDI	From 500 to 1,000	*Brachydanio rerio*	OECD 203, static, 96 hours, no analytical monitoring, MDI dispersed by slight stirring with a glass rod.	$LC_0 \geq 1,000$	-	2	(Caspers et al., 1986)
Polymeric MDI	3,000	*Oryzias latipes*	Nichi-Nou-Sei B2735 (1965)*, semistatic, 48 hours, no analytical monitoring, MDI dispersed by stirring.	$LC_0 \geq 3,000$	-	2	(Nakata, 1983)
Polymeric MDI	3,000	*Oryzias latipes*	Nichi-Nou-Sei B2735 (1965)*, semistatic, 72 hours, no analytical monitoring, MDI dispersed by stirring.	$LC_0 \geq 3,000$	-	2	(Nakata, 1983)
Polymeric MDI	3,000	*Oryzias latipes*	Nichi-Nou-Sei B2735 (1965)*, semistatic, 96 hours, no analytical monitoring, MDI dispersed by stirring.	$LC_0 \geq 3,000$	-	2	(Nakata, 1983)

* Test method similar to OECD Test Guideline 203

Table 3.7 MDI toxicity to fish: long-term studies.

Long-term studies							
Substance tested	Concentrations tested (mg/l)	Species	Method	Endpoint (mg/l)	MDA analyses	Reliability index	Reference
Polymeric MDI	0.1	*Cyprinus carpio*	8 weeks in river model ecosystem, no analytical monitoring	No mortality observed with 0.1 mg/l	-	3	Fujiwara, 1981
Polymeric MDI	1,000 and 10,000	*Onchorhynchus mykiss*	112 days in experimental ponds; MDI added directly on the sediment with a hose; virtually no MDI detected in water during experiment (detection limit: c.a. 0.01 mg/l)	Weight reduction leading to death of half of the test fish with 10 g MDI/l due to decrease of cladoceran abundance in the pond	no MDA detected in water during experiment (detection limit: c.a. 0.01 mg/l)	2	Heimbach et al., 1996

No lethal effect has been observed in short-term studies even with nominal concentrations of 3,000 mg/l. Although the authors of the studies did not check the MDI concentrations at the end of the test period it is most likely that MDI disappeared from the media after addition to the water (see Section 1.3.6 water solubility) and so it is probable that the absence of an effect in the tests was due to the instability in water of MDI and the inertness of its hydrolysis products.

It should also be noted that even when MDA (methylenedianiline) was formed during the experiments, the levels of this product in the media must have been very low as toxic effects to the animals were not noted. The MDA concentration in the medium of acute toxicity tests was only measured in one study (Rhône-Poulenc, 1977). An initial concentration of 500 mg/l of MDI added to the fish medium by stirring for 18 hours yielded concentrations of, respectively 3.5 mg/l and 4 mg/l of MDA for polymeric MDI or 4,4'-MDI after a 24 hours incubation period. As this MDA level is at least ten times lower than the LC_{50} of MDA for fish (from 32 to 65 mg/l, Risk Assessment Report MDA (EC, 2000)) no mortality to tests organisms was observed. Caspers and co-workers (1986) reported a markedly increased toxicity to fish when MDI was dispersed into the experimental medium by high speed shearing, although the data obtained were not reported by the authors because they were inconsistent, the increased toxicity might have been caused by an increased MDA yield in the medium. Nevertheless the results are probably specific for this dispersing method and do not reflect situations which might occur in the environment.

No direct toxic effects have been observed in long-term studies with nominal concentrations of up to 10 g/l. But it must be noted that indirect impact on fish through decrease of their natural food (cladocerans) in an artificial pond to which was added 10 g/l of polymeric MDI was observed by Heimbach et al. (1996).

3.2.1.2 Aquatic invertebrates

The MDI toxicity studies with aquatic invertebrates are presented in **Table 3.8** and **Table 3.9**.

Table 3.8 MDI toxicity to aquatic invertebrates: short-term studies.

Short-term studies							
Substance tested	Concentrations tested (mg/l)	Species	Method	Endpoint (mg/l)	MDA analyses	Reliability index	Reference
4,4' MDI	500	*Daphnia magna*	AFNOR T.90.301 April 74, 24 hours, no analytical monitoring, MDI dispersed by magnetical stirring for 18 hours.	$EC_0 \geq 500$ (mobility)	-	3	Rhône-Poulenc, 1977
Polymeric MDI	500	*Daphnia magna*	AFNOR T.90.301 April 74, 24 hours, no analytical monitoring, MDI dispersed by magnetical stirring for 18 hours.	$EC_0 \geq 500$ (mobility)	-	3	Rhône-Poulenc, 1977
4,4' MDI	500	*Limnea stagnalis*	Other, 24 hours, no analytical monitoring, MDI dispersed by magnetical stirring for 18 hours.	$EC_0 \geq 500$ (embryos and young)	-	3	Rhône-Poulenc, 1977
Polymeric MDI	500	*Limnea stagnalis*	Other, 24 hours, no analytical monitoring, MDI dispersed by magnetical stirring for 18 hours.	$EC_0 \geq 500$	-	3	Rhône-Poulenc, 1977
Polymeric MDI	from 0.5 to 500 from 20 to 1000	*Daphnia magna*	OECD 202, 24 hours, no analytical monitoring, MDI dispersed by - high speed shearing for 60 seconds - Magnetic stirring for 30 minutes	EC_{50}: 129.7 EC_{50} > 1,000	- -	2	Caspers et al., 1986

Table 3.9 MDI toxicity to aquatic invertebrates: long term studies.

Long-term studies							
Substance tested	Concentrations tested (mg/l)	Species	Method	Endpoint(mg/l)	MDA analysis	Reliability index	Reference
Polymeric MDI	From 1 to 10	*Daphnia magna*	OECD 202 part 2, MDI dispersed by slight stirring with a glass rod.	21 day reproduction not affected with 10 mg/l	-	2	Caspers et al., 1986
Polymeric MDI	1,000 and 10,000	Copepods Cladocerans Rotifers Oligochaetes Gasteropods Bivalves Dipter larvae	112 days in experimental ponds; MDI added directly on the sediment with a hose; virtually no MDI detected in water during experiment (detection limit: approximately 0.01 mg/l)	Copepods /cladocerans: less abundant from week 2 to week 8. Rotifers: more abundant at week 2 and week 16. Gasteropods unaffected. All benthic organisms affected by physical obstruction of their habitat and anoxia but all taxa recovered by the end of the study with the exception of bivalves which have too long generation times	no MDA detected in water during experiment (detection limit: approximately 0.01 mg/l)	2	Heimbach et al. 1996

No lethal effects have been observed in short-term studies even with nominal concentrations of 1,000 mg/l with the exception of one study (Caspers et al., 1986) where MDI was dispersed into the medium by high speed shearing instead of usual stirring; this must have led to an increased production of MDA (methylenedianiline) which could be responsible of the lethal effect caused to the daphnids since daphnids are quite sensitive to MDA as indicated by the relatively low EC_{50} for *Moina macropa* (2.3mg/l; Risk Assessment Report MDA (EC, 2000)). As stated in Section 3.2.1.1., the authors considered these data as irrelevant because the dispersing method does not reflect a situation that might happen in the real environment.

Long-term studies show that MDI has only indirect effects on aquatic invertebrates. However, the potential negative impact on benthic organisms of the polyurea crust resulting from the reaction of MDI with water should be considered. In the study reported by Heimbach et al. (1996) there was a physical effect noted on the benthic organisms. On a local scale an accidental spill would have a dramatic effect on those organisms. However, if the crust is removed from the sediment as a restoration measure, a re-colonisation of the covered area by animals from the surroundings will most probably occur within a short time.

3.2.1.3 Algae

The MDI toxicity studies with algae are presented in **Table 3.10**.

Table 3.10 MDI toxicity to algae.

Substance tested	Concentrations tested(mg/l)	Species	Method	Endpoint (mg/l)	MDA analysis	Reliability index	Reference
Polymeric MDI	Range-finding test: from 3 to 1,070	*Scenedesmus subspicatus*	OECD 201; 3 days; MDI stirred for 1 hour in medium and clear upper layer siphoned off and used for tests; no analytical monitoring	Limit test 1640 mg/l (no effect observed)	-	2	(Blom and Oldersma, 1994)
Polymeric MDI	1,000 and 10,000	Phytoplankton of artificial outdoor ponds	112 days, artificial ponds; up to 10 mg/l added to the water, no MDI nor MDA detected in the water during the experiment	Minor effects due to the CO_2 liberation were observed (chlorophytes more abundant and other taxa less abundant).	no MDA detected in water during experiment (detection limit: approximately 0.01 mg/l)	2	(Heimbach et al. 1996)
Polymeric MDI	1,000 and 10,000	Macrophytes	112 days in experimental ponds; MDI added directly on the sediment with a hose; virtually no MDI detected in water during experiment (detection limit: approximately 0.01 mg/l)	Less numerous due to physical obstruction of emergence sites but nevertheless the biomass increase is greater (CO_2 emissions).	no MDA detected in water during experiment (detection limit: approximately 0.01 mg/l)	2	(Heimbach et al. 1996)

No significative negative impact of the MDI was observed towards algae except the physical hindrance of macrophyte emergence due to the polyurea solid crust formation. As an indication of the potential indirect hazard due to MDA (methylenedianiline) formation: the EC_{50} for *Scenedesmus subspicatus* (72 hours) is 21 mg/l as determined in a separate, similar test where MDA toxicity to this alga was tested (see RAR MDA (EC, 2000)).

3.2.1.4 Microorganisms

The MDI toxicity studies with microorganisms are presented in **Table 3.11**.

Table 3.11 MDI toxicity to microorganisms.

Substance tested	Concentrations tested (mg/l)	Species	Method	Endpoint (mg/l)	MDA analysis	Reliability index	Reference
Polymeric MDI	100	*Escherichia coli*	10 days, inhibition growth, no analytical monitoring.	$EC_0 \geq 100$	-	3	Fujiwara, 1981
Polymeric MDI	From 1 to 100	Activated sludge	OECD 209, Respiration inhibition test, MDI dispersed by slight stirring with a glass rod.	$EC_{50} > 100$	-	2	Caspers et al., 1986

No toxic effect of MDI was observed on microorganisms but as stated before it is most likely that MDI disappeared from the test media very briefly after addition to the aqueous media (see Section 3.2) and so it is not possible to determine if the absence of the effect in the performed test is due to true innocuousness of the tested substance or rather to its particular instability in water.

3.2.2 Atmosphere

The very low vapour pressure of MDI at ambient temperatures makes it very difficult to generate an atmosphere with sufficient concentration to cause toxic effects. The results of inhalation studies reported below, performed with rats, were obtained by artificially generating aerosols, not representative of normal handling and use of MDI. This is discussed more fully in Section 4.

Acute toxicity (LC_{50}): see Section 4.1.2.2 and **Table 4.12**.

- 0.172-0.187 mg 4.4'-MDI/ l of air (1 hour of exposure) (Wazeter, 1965)
- 0.49 mg polymeric MDI/l of air (4 hours) (Appelman, 1982a)
- 0.369 mg MDI (homologous mixture)/l of air (4 hours) (Bunge et al., 1977)

Repeated dose toxicity: see Section 4.1.2.6 and **Table 4.32**

- NOAEL > $26.8.10^{-3}$ mg/l of air (polymeric MDI, male rats, 2 weeks of exposure) (Wazeter, 1964e)
- NOAEL >$2.9.10^{-3}$ mg/l of air (polymeric MDI, female rats, 4 weeks of exposure) (Wazeter, 1964b)
- NOAEL < 4.10^{-3} mg/l of air (polymeric MDI, 13 weeks of exposure) (Reuzel et al., 1986)

- NOAEL = $0.2.10^{-3}$ mg/l of air (polymeric MDI, up to 2 years of exposure; monomeric 4,4'-MDI, 2 years exposure) (Reuzel et al., 1990; Hoymann et al., 1995)

Carcinogenicity: Section 4.1.2.8 and Section 4.1.2.8.4.1

- NOAEL = $0.2.10^{-3}$ mg/l of air (polymeric MDI, up to 2 years of exposure; monomeric 4,4'-MDI, 2 years exposure) (Reuzel et al., 1990; Hoymann et al., 1995)
- Toxicity for reproduction: see Section 4.1.2.9 and **Table 4.39**
- NOAEL for developmental toxicity = $4. 10^{-3}$ mg/l of air (polymeric MDI) (Gamer et al., 2000)
- NOAEL for developmental toxicity = $3. 10^{-3}$ mg/l of air (monomeric 4,4'-MDI) (Bushmann et al., 1996)

So it is assumed that MDI can cause toxic effects to rats when concentrations above $0.2.10^{-3}$ mg/l of air are considered

For further details see Sections 4.

3.2.3 Terrestrial compartment

The MDI toxicity studies with terrestrial organisms are presented in **Table 3.12**.

Table 3.12 MDI toxicity to terrestrial organisms.

Substance tested	Concentrations tested (mg/kg dw soil)	Species	Method	Endpoint (mg/kg dw soil)	MDA analyses	Reliability index	Reference
Mixture of polymeric and monomeric MDI	1,000	*Eisenia fetida*	OECD 207, 14 days, no analytical monitoring; MDI coated on sand through an acetone bath and drying for 2 days.	No effect observed with up to 1,000 mg/kg of artificial soil.	-	2	(Van der Hoevan, 1992b)
Polymeric MDI	10, 100, 1,000 (range-finding test)	*Avena sativa*	OECD 208, 14 days, no analytical monitoring; MDI coated on sand through an acetone bath and drying under N_2 stream.	EC_{50} > 1,000	-	2	(Van der Hoevan, 1992a)
Polymeric MDI	10, 100, 1,000 (range-finding test)	*Lactuca sativa*	OECD 208, 14 days, no analytical monitoring; MDI coated on sand through an acetone bath and drying under N_2 stream.	EC_{50} > 1,000	-	2	(Van der Hoevan, 1992a)

No toxic effect of MDI was observed on the terrestrial organisms tested but it must be stated that in the tests performed, MDI was in contact with water (in moist soils) and so it is very likely that the MDI concentrations tested were much lower than the nominal ones mentioned by the authors and one should be very cautious about the conclusions that can be drawn from the data available. As an indication of the potential indirect hazard due to MDA (methylenedianiline) formation the EC_{50} for *Eisenia fetida, Avena sativa* and *Lactuca sativa* are respectively of 444, 353 and 128 mg/kg dw soil as determined in separate, similar tests where MDA toxicity to these organisms was tested (see RAR MDA, (EC, 2000)).

3.2.4 Non compartment specific effects relevant to the food chain

Two studies are reported where the potential accumulation of MDI in aquatic systems was tested. In the study by Heimbach et al. (1996) up to 10 g of polymeric MDI was added per litre of water into artificial outdoor ponds and the concentration of MDI was monitored for 112 days in all compartments (water, fish (*Oncorhynchus mykiss*), and sediment). No traces of MDI or MDA (methylenedianiline) in water and fish throughout the whole experimentation period were detected; the detection limit of MDI and MDA in water was of 5-10 µg/l and 1.45 µg/l and of 0.5 mg/kg for MDA and MDI monomer in fish. In another study (Fujiwara, 1981) the fish *Cyprinus carpio* did not accumulate any MDI (detection limit 0.1 mg/kg) after 8 weeks in a river model to which was added 0.1 mg/l of polymeric MDI. These studies give evidence that MDI accumulation through aquatic food chains is very unlikely as might be expected considering the very low solubility of MDI in water.

3.2.5 Predicted no-effect concentrations (PNECs)

The impossibility of performing any aquatic ecotoxicological test in the absence of water implies that derivation of PNECs for MDI from the results mentioned in this section is very difficult. Indeed, it is recommended in test guidelines to keep the concentration in experimental media above 80% of the nominal concentration. When attempts were made to measure MDI concentrations in experimental media, the results were always below the detection limits. Nevertheless, PNECs calculated on the basis of nominal concentrations of MDI could be considered as the amount of product that can be added per volume of media above which an effect will not occur. We chose to opt for this strategy rather than to use the solubility limit for $PNEC_{aqua}$ as water solubility of MDI is a notional concept because of its transient nature in aqueous media (see Section 3.2.1). The value reported for water solubility of MDI has thus no quantitative relevance to infer $PNEC_{aqua}$. Furthermore given the reactivity of MDI with water and the subsequent formation of the inert polyureas, it is probably more suitable for this assessment to only use acute data. We are aware that designation of the following effect thresholds by PNEC is not consistent with the approach adopted for non-reactive chemicals.

Only $PNEC_{aqua}$ and $PNEC_{soil}$ could be calculated on the basis of the available effect data. Data and assessment factors are as follows:

3.2.5.1 Aquatic compartment

Table 3.13 MDI toxicity to aquatic organisms

Test organism	Endpoint	Value
Fish	Acute/short-term: EC_{50}	from > 1,000 to > 3,000 mg/l
Daphnid	Acute/short-term: EC_{50}	> 1,000 mg/l
	Reproduction/long-term: NOEC	>10 mg/l
Algae	72 hours: EC_{50}	>1,640 mg/l
	NOEC	>1,640 mg/l

All tests performed were limit tests and consequently no concentration-effect relationship could be established. Consequently NOECs were not considered for calculating the PNEC as advised in the TGD.

Assessment factor: 1,000 (for three short term studies)

PNEC: > 1,000 mg.l^{-1}/1,000 = > 1 mg/l

3.2.5.2 Terrestrial compartment

Table 3.14 MDI toxicity to terrestrial organisms

Test organism	Endpoint	Value
Earthworm	14 days: EC_{50}	> 1,000 mg/kg
Plant	14 days: EC_{50}	> 1,000 mg/kg

Assessment factor: 1,000

PNEC: > 1,000 mg.kg^{-1}/1,000 = > 1 mg/kg

3.2.5.3 Sediment compartment

A PNEC value for sediment may be calculated using the equilibrium partitioning (EQP) approach; the value obtained is 108 mg/kg wet weight. Nevertheless, this method is based on extrapolations directly based on effect data for aquatic organisms and the partition coefficient of the substance between octanol and water. Given the ease of hydrolysis of MDI, effect data are to be used with great caution and concepts of water solubility and partition are not meaningful. As a consequence, we do not provide details of PNECsediment calculation and consider it is not realistic to infer the real behaviour of MDI in aqueous media on the basis of such data.

It is to be noted, though, that effects of MDI on benthic organisms of artificial outdoor ponds were studied by Heimbach (1993). This author noted that benthic species that are mobile are not affected by an addition of up to 10g of MDI per litre to a 4.5 m^3 pond. Among sessile taxa, only those with long generation times (bivalves) did not recover from the simulated spill. These results tend to indicate that, in the case of non accidental releases of MDI to the environment, no disruption of MDI to benthic organisms is expected, especially so as in this case (non accidental release) the MDI load in water will be much lower than 10g l^{-1}.

3.3 RISK CHARACTERISATION

3.3.1 Aquatic compartment

PNEC for the **aquatic organisms** is 1.0 mg/l, since all regional and local PECs in surface water are lower than 1.0 mg/l (maximal PEC is $1.4\ 10^{-6}$ mg/l) all PEC/PNECs will be lower than 1 and it can be considered that MDI will not lead to environmental hazards for aquatic organisms.

3.3.2 Terrestrial compartment

PNEC for the **terrestrial compartment** is 1 mg/kg WWT, since both regional and local PECs in soil are lower than this value (maximal PEC is $7.22\ 10^{-4}$ mg/kg WWT) all PEC/PNEC will be lower than 1 and it can be considered that MDI will not lead to environmental hazards for terrestrial organisms either.

All risk characterisation ratios calculated by the EUSES program are presented hereafter:

Table 3.15 Risk characteristics ratios for the terrestrial compartment calculated by EUSES

Compartment	Production	Processing to PU	Processing to prepolymers	Processing of prepolymers speciality MDI's	Processing of prepolymers other than speciality MDI's	Regional
Water	$<1.37\ 10^{-6}$	$<1.37\ 10^{-6}$	$<1.37\ 10^{-6}$	$<1.37\ 10^{-6}$	$<1.37\ 10^{-6}$	$<1.37\ 10^{-6}$
Soil	$<5.36\ 10^{-5}$	$<4.78\ 10^{-4}$	$<1.83\ 10^{-4}$	$<1.37\ 10^{-4}$	$<2.35\ 10^{-4}$	$<4.21\ 10^{-5}$
Sediment	$<1.54\ 10^{-6}$	$<1.54\ 10^{-6}$	$<1.54\ 10^{-6}$	$<1.54\ 10^{-6}$	$<1.54\ 10^{-6}$	$<6.4510^{-7}$
Sewage treatment plants	0	0	0	0	0	-

3.3.3 Sewage treatment plants

The EUSES program predicts that MDI will not induce perturbations to **sewage treatment plants.** This conclusion is obvious since releases of MDI by production and big processing plants (> 76% of tonnage) to STP are virtually non-existent. Moreover, any traces of MDI rejected into the water would undergo hydrolysis and disappear from effluent water very rapidly.

3.3.4 Atmospheric compartment

The **atmospheric compartment** was not included in the quantitative risk assessment because of the lack of information relevant to this compartment

3.3.5 Secondary poisoning

Secondary poisoning is very unlikely to occur considering the results of accumulation studies with MDI (see Section 3.1.4).

As explained in Section 3.1.2, MDA (methylenedianiline) is a minor product of environmental contact of MDI with water. The following brief section considers the possible extra exposure to

any MDA that might be produced by environmental release of MDI. The procedure is to assume a rather high value for the degree of conversion of MDI to MDA in aqueous media and compare the resulting PEC values for MDA with those quoted in the MDA RAR (EC, 2000).

As has been exposed in Section 3.1.2 yields of MDA when MDI is added to aqueous media are comprised between 0.128 and 2%.

If a yield of 2% is considered (worst case), Local PECs of MDA associated with MDI hydrolysis are then as follows:

Table 3.16 Local PECs

Environmental compartment	Production	Processing to PU	Processing to prepolymers	Processing of prepolymers speciality MDI's	Processing of prepolymers other than speciality MDI's
Surface water (mg/l)	$2.8\ 10^{-8}$ (*)	$2.8\ 10^{-8}$	$2.8\ 10^{-8}$	$2.8\ 10^{-8}$	$2.8\ 10^{-8}$

(*) MDI PECs in surface water (see Table 3.3) i.e. $1.37\ 10^{-6}$ · 2%

If these concentrations are compared to those reported in the MDA RAR Report (C_{local} in surface water = 69.10^{-3} mg/l for the generic approach and $0.4.10^{-3}$ for the site-specific approach), it appears that they are **many orders of magnitude** less than the PEC values quoted in the RAR on MDA (EC, 2000). Adding them to the C_{local} calculated without considering MDA releases due to MDI production and processing will not change the risk characterisation ratios that are as follows:

Table 3.17 RCR for generic and site-specific approaches

	RCR
Generic approach	23
Site-specific approach Worst case	0.13

(PNEC aqua of MDA being 3 µg/l)

Since the RCR for MDA in the site-specific/worst-case approach is already <1 it can also be understood that no environmental hazard is expected from MDA derived from MDI degradation in water.

4 HUMAN HEALTH

4.1 HUMAN HEALTH (TOXICITY)

It is important to mention that, in general, there is often a lack of transparency with regard to doses or concentrations used in the experimental toxicity studies and in the exposure assessment, particularly in some of the older studies, thus making a quantitative risk assessment uncertain. This problem is inherent to MDI, because of its different forms – monomers, prepolymers, polymers, vapours, aerosols and dusts – which have different physico-chemical properties, possibly different absorption characteristics for dermal or inhalation exposure, and, therefore, different exposure-toxicity relationships.

In the present assessment all exposures are expressed in mg/m³, although the figures in the original publications are often given as parts per million (ppm). Strictly, the use of ppm is only appropriate for compounds in pure gaseous forms and the conversion between ppm and mg/m³ may be done using the formula "mg/m³ = ppm · molecular weight / 24.45", based on the law of perfect gases. In the following, all ppm figures have been converted to mg/m³, even in the case of aerosols, using the following approximation:

$$\text{mg MDI/m}^3 = \text{ppm} \cdot 250.26 / 24.45 \approx \text{ppm} \cdot 10$$

4.1.1 Exposure assessment

4.1.1.1 General discussion

MDI is mainly used in the production of rigid polyurethane foams; however, there are also many applications in the fields of Coatings, Adhesives, Sealants and Elastomers (CASE) such as paints, adhesives, weather resistant sealing materials and footwear. MDI is also used in the production of particle board and mould cores for the foundry industry.

In rigid polyurethane production, low density products (30-50 kg/m^3) are mainly used for insulation purposes such as insulation panels, spray foam on walls and roofs, refrigerator insulation, oil storage tanks, refrigerated container transport and car accessories.

The estimated percentage MDI usage in various applications is given in **Table 2.4**.

The Danish Product Register (June 1998) has supplied data on the quantity of MDI containing industrial products. The number of products and quantity of the substance (MDI) are distributed according to concentration intervals. In the following table "Nbr." states the number of products containing MDI within the given concentration interval. The quantity of MDI in these products is given in tons per annum rounded to the nearest integer of tons. The number of products with no data on quantity or MDI concentration are stated in a separate line marked n.d. Finally, the total number of products containing MDI and the total quantity of MDI are stated.

Following this the most important product types are listed based on statements from manufacturers according to their knowledge and estimate.

Substance	Nbr.	Quantity t/a
MDI		
0 – 1%	397	15
1 – 5%	123	64
5 – 10%	97	868
10 – 20%	102	110
20 – 50%	238	5,109
50 – 80%	187	4,238
80 – 100%	96	6,608
n.d.	21	
Total	1,261	17,012

Product types

Process regulators (hardeners)		
0 – 1%	11	<1
1 – 5%	24	20
5 – 10%	10	13
10 – 20%	25	48
20 – 50%	103	1,923
50 – 80%	125	3,758
80 – 100%	62	2,977
Total	360	8,739

Construction materials		
0 – 1%	4	<1
5 – 10%	3	7
10 – 20%	11	1
20 – 50%	18	2,299
50 – 80%	29	958
80 – 100%	17	3,503
Total	82	6,768

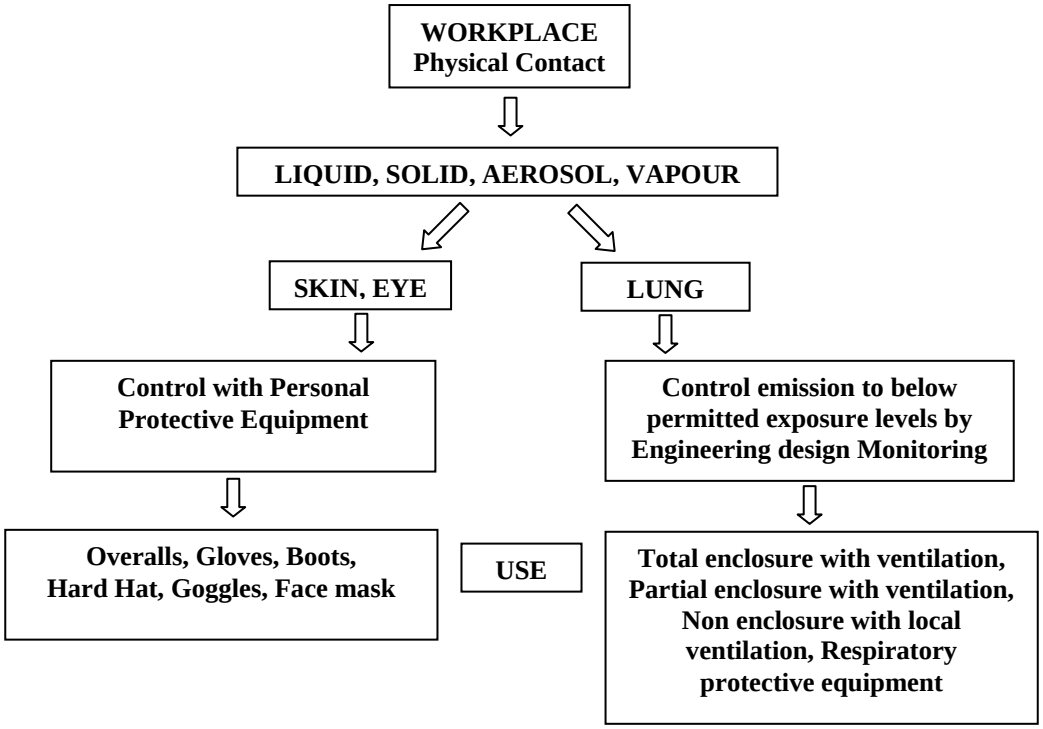
Insulation materials/foaming agents		
10 – 20%	4	8
20 – 50%	12	1,985
50 – 80%	6	1,666
Total	22	3,662

Intermediates		
Total	8	2,696

Occupational exposure may occur by inhalation of vapours and aerosols or through skin exposure at workplaces where MDI is produced or used. Inhalation can also occur with dust arising from the handling of pure MDI (solid at ambient temperatures).

Airborne MDI monomer can be either a vapour, an aerosol, or a combination of the two. The polymeric, prepolymeric, modified, or partially polymerised MDI will exist primarily as aerosols, not vapours, due to its higher molecular weights. However, polymeric, prepolymeric, modified, or partially polymerised MDI is never sprayed in its original form, but usually mixed with a reacting species i.e. polyol, and hence such reactive aerosols have a rapidly decreasing MDI content.

Figure 4.1 Potential for exposure to MDI and current use of protective measures during manufacturing and use of the substance (according to industry)



However, PPE should not be identified as the first choice for skin and eye protection from exposure to MDI. In order of preference, considerations should be given to:

1) enclosing or modifying the process to isolate the hazard source;

2) installing appropriate local exhaust ventilation close to hazard source;

3) changing working methods to exclude or restrict access to the hazard source;

4) issuing PPE to workers to protect them from the hazard source.

Typical 'permissible' workplace exposure levels for MDI in Europe are:

Belgium	0.05 mg/m^3 OEL
France	10 ppb VME (0.1 mg/m³)
	20 ppb VLE (0.2 mg/m³)
Germany	5 ppb MAK (0.05 mg/m³)
Italy	5 ppb TLV-TWA (0.05mg/m³)
The Netherlands	0.05 mg/m^3 MAC
UK	0.02 mg NCO/m^3 MEL-TWA
	0.07 mg NCO/m^3 MEL-STEL

However, 'permissible workplace exposure level' is a misleading term. No completely 'safe' level of exposure could be identified for diisocyanates. The industry is obliged to control workplace exposure to diisocyanates to as low as is reasonably practicable and, in any case, below the 'permissible workplace exposure level'.

Concentrations of isocyanates can vary considerably. It appears from the available data received from industry and other sources that MDI exposure in the working environment is normally below the national occupational standards, but high exposure to MDI may occur if an aerosol/particulate of MDI is formed. This is the case, for example, during spray painting using MDI containing products. An important consideration when monitoring is that it should follow as closely as possible the exposure of the workers whilst carrying out their normal work operations.

At present a number of analytical methods and techniques are available for sampling and analysing isocyanates in workplace atmospheres. Great care must be taken to ensure that a representative sample is collected. The occupational hygienist or the environmental analyst must consider the physical state of the MDI likely to be present in the workplace air being sampled: for example, will the isocyanate be present as a vapour and/or condensation aerosol, or will it be coated on another medium. All this must be considered when selecting a method for monitoring workplace exposures.

Many laboratories use in-situ derivatisation methods for the determination of MDI in workplace air, for example, OSHA 47, MDHS 25/3, etc. The aforementioned methods use either glass impingers containing a solution of either 1-(2-pyridyl) piperazine (OSHA 47) or 1-(2-methoxyphenyl) piperazine (MDHS 25/3) derivatising reagents in toluene or fibre glass filters impregnated with the same reagent or a combination of bubbler and filter, which will collect both vapour and aerosol/particulates of MDI. The principle of the methods requires a measured volume of air to be drawn through the sampling device at a constant rate. Airborne MDI reacts with the derivatising reagent in the impinger or on the filter to form a stable non-volatile urea derivative. Following subsequent extraction and preconcentration stages, analysis is performed using high performance liquid chromatography (HPLC) with an ultraviolet (UV) detector. In addition, an electrochemical (EC) detection system can be used in series with the UV detector when using MDHS 25/3 to measure the presence of polymeric MDI or other "NCO" species. Finally, the MDI-derived peaks are qualitatively identified by their retention times and quantitatively determined by their peak areas.

An alternative method, proposed by Skarping and his group, uses di-n-butylamine as reagent and gas chromatography-mass spectrometry methods (GC-MS) for detection. It is the recommended

method for measuring isocyanates in Sweden. At the Swedish Institute for Working Life in Umeå, research is in progress on the MDHS 25/3 method using mass spectrometric detection instead of UV and EC detection.

However measurement of air levels alone is probably not sufficient for MDI monitoring, as its potential uptake through the skin and individual differences in metabolism would not be considered. Recently, colorimetric sampling pads were developed. These pads qualitatively detect surface and skin contamination of aliphatic and aromatic isocyanates by changing colours when in contact with specific isocyanates. The sampling tool has been recommended by Occupational Safety and Health Administration (OSHA), Salt Lake City Technical Center, Utah for evaluation of surface and skin contamination by isocyanates in auto body shops (OSHA, 1999).

There is a need for sensitive biological markers of recent and long-term exposure (Sepai et al., 1995a). Biomonitoring of MDI based on its degradation product MDA has been considered. This would have the advantage that it might be possible to assess dermal exposure and the exposure many days after the exposure (Skarping et al., 1995). Further, a biological monitoring method could have great potential for estimating the individual exposure to MDI as all the routes of exposure are taken into account. Skarping and Dalene (1995) tried to demonstrate the efficiency of the GC-MS method for MDI and MDA biomonitoring in urine and plasma. However, no validated MDA-biomonitoring method has been published yet. The Deutsche Forschungsgemeinschaft (D.F.G.) is still writing a method.

A workshop was held in Brussels 26-28 April 1999, 'Isocyanates: Measurements, Methodology, Exposure and Effects' with about 20 occupational experts in this field. This meeting was an up to date summary of the knowledge in the area. A state of the art document from this workshop was published in the 'Worklife 2000, Yearbook 1999', edited by Richard Ennals, published by Springer.

4.1.1.2 Occupational exposure

The following data are used for occupational assessment:

- physico-chemical data, physical appearance and vapour pressure at room temperature, percentage of MDI in products
- data regarding the production process and use pattern
- exposure data from industry
- exposure data from HEDSET and literature
- results from exposure models (EASE-model, EUSES, SKINPERM)

The model for the Estimation and Assessment of Substance Exposure (EASE), developed as part of the guidance on new and existing substances (TGD, 1996) has been used to predict occupational exposure.

In a first attempt to make an estimation of the skin permeation, the SKINPERM program (version 9.01, 1998) was run. However, the results of the modelling have been placed in Annex 2, as reliable *in vivo* study results (Leibold et al., 1998) have become available and these are used in the ultimate assessment.

Moreover, the exposure is assessed without taking account of the possible influence of personal protective equipment (PPE). If the assessment as based on potential exposure indicates that risks are to be expected, the use of PPE may be one of the methods to decrease actual risks, although other methods (technical and organisational) are to be preferred. This is in fact obligatory following harmonised European legislation.

The following measures have been proposed as guidance:

Knowledge of the efficacy of PPE in practical situations is very limited. Furthermore, the efficacy is largely dependent on site-specific aspects of management, procedures and training of workers. For respiratory protection the efficiency depends largely on the type of protection used. Without specific information, a tentative reduction efficiency of 90% may be assumed, equivalent to the assigned protection factors for supplied-air respirators with a half mask in negative pressure mode (NIOSH, 1987). Better protection devices will lead to higher protection. Imperfect use of the respiratory protection will lower the practical protection factor compared to the assigned factor. These estimations of reduction are not generally applicable "reasonable worst case" estimations, but indicative values based on very limited data. They will not be used directly in the exposure and risk assessment.

The PPE is specified for all break-ins (planned access to sealed plant such as vessels and pipe work, etc.) and is determined by temperature of the operation. This includes chemically protective suits, gloves, aprons, safety shoes, overalls and self-contained breathing protection if no adequate local exhaust is installed. Eye protection should be worn at all times when handling and operating machines using MDI. Isocyanates tend to harden rubber and most plastics and increase the risk of their splitting. Gloves should be replaced as soon as there is any significant hardening. In general, the use of disposable gloves and clothing is often preferred, because proper decontamination of reusable items is often difficult.

In a report submitted to the International Isocyanate Institute (III), by TRI/Environmental, Inc. (1996), 32 different candidate gloves, representing nine different glove polymer types, were evaluated in a permeation testing of protective glove materials with MDI and TDI in production solvents. As MDI/chlorobenzene solutions are used in MDI producing factories (in closed systems), solutions of 10% and 90% MDI in chlorobenzene were tested. The permeation tests were performed by selective detection of the isocyanates through an analytical method, which was a modification of the Marcali method for detecting isocyanates in the air. The results showed that the performance of the gloves depended on the concentration of the chlorinated solvent. There were many more gloves that performed well in 10% solvent than in 90% solvent. Ultimately, in 90% solvent, there were only three gloves identified that would protect a worker from exposure over an eight-hour shift. These were North Silver Shield and Safety 4 4H (formerly Ansell Edmont), both laminated polyethylene, and North Viton F101, a fluoroelastomer. On the other hand, if the chlorinated hydrocarbon concentration is low, butyl rubber, nitrile rubber, and some thicker neoprene gloves offer adequate protection.

The exposure is assessed using the available information on substance, processes and work tasks. However more detailed information on these parameters is needed to make a more accurate exposure assessment.

For the occupational exposure assessment the exposure situations can be clustered into different scenarios based upon the type of process and activity and the possibilities for exposure that relate to that process and activity. In the first scenario the production of MDI and prepolymers in almost completely closed systems is considered. The second scenario considers the use of MDI

as an intermediate by the downstream users. This scenario considers the use of almost completely closed systems but also the use of MDI as an aerosol in spraying applications.

4.1.1.2.1 Scenario 1: Chemical industry, the production of MDI and prepolymers

The production of MDI is an advanced chemical process. MDI is produced commercially from aniline and formaldehyde as starting materials, using hydrochloric acid as catalyst. This condensation reaction produces 4,4'-methylenedianiline (4,4'-MDA) and a complex mixture of polyamines, which are then phosgenated to obtain a methylene diphenyl diisocyanate mixture. In this continuous phosgenation process, the process temperature rises up to 150 – 200°C.

Industrial production of MDI does not produce pure isomers, but a mixture primarily containing 4,4'-isomer, with isomer proportions varying according to the exact process used. The production material has by common usage been given the name generic MDI.

Monomeric 4,4'-MDI is produced only by distillation of the isomer mixture. The 2,4'- and 2,2'-isomers are very difficult to isolate.

Polymeric MDI is made by the phosgenation process, but it contains a significant excess of monomeric MDI remaining – typically around 50% and a specified –NCO content of 31.5 ± 1%.

The MDI prepolymers may be based on pure MDI, mixtures of MDI isomers or on MDI oligomers "polymeric MDI". The di-isocyanates are partly reacted with hydroxy compounds to give a mixture of compounds which are terminated in isocyanate or blocked isocyanate groups. These prepolymers are subsequently used in the manufacture of polyurethanes by further reaction with polyols.

The production of MDI involves the use of closed systems. Process control is mainly automated via a central control room, so that the potential for worker exposure is minimised. The batch synthesis of isocyanate prepolymers uses the output of the MDI plant as raw material at the same chemical manufacturing site. Final products are stored in tanks and despatched by pipeline and road tankers. Exposure is therefore likely to be intermittent and may occur during sampling and analysis, during filter changing, during connecting and disconnecting pipelines, during loading and unloading road tankers, filling and drumming, during repair, maintenance and cleaning activities (e.g. hydroblasting), vessel entry, stack venting, during process upsets at systems or units with higher temperatures, during accidental spills.

Inhalation exposure

Measuring results

The results of workplace measurements in the field of production and processing in the chemical industry submitted by several companies are presented in **Table 4.1**:

Table 4.1 Workplace measurements from the chemical industry

Job category / activities	Year of measurement	Number of samples	Range of measurement data (mg/m³)	Median (mg/m³)	95% percentile	Techniques / sampling used
MDI production	1992-1996	60	< 0.002 – 0.020	< 0.002	Not relevant [1]	Personal 8-hour sampling: MDHS-25 method
Prepolymer plant	1992-1996	16	< 0.002 – 0.022	< 0.002	Not relevant [1]	Personal 8-hour sampling: MDHS-25 method
Laboratories	1992	78	< 0.001 – 0.030	< 0.002	Not relevant [1]	Personal 8-hour sampling: MDHS-25 method
MDI production	1995	152	< 0.002	< 0.002		Personal 8-hour sampling: MDHS-25 method
MDI production	1996	144	< 0.0005 – 0.024	< 0.0005		Personal 8-hour sampling: MDHS-25 method
MDI production	1997	73	< 0.0005 – 0.003	< 0.0005		Personal 8-hour sampling: MDHS-25 method
MDI production	1987-1996	22	-	0.01 [2]		Not known
MDI production	1989-1996	8	-	< 0.01 [2]	≤ 0.01 (90-percentile)	Not known
MDI production	1995	6	≤ 0.0005 [2]			Not known
MDI production	1997	3	< 0.09 [3]			Not known
Polymeric MDI production	1986-1987	49	V.D. = 0.2 [4]	0.02	0.01	GMD MDI 901 Autostep
Polymeric MDI production	1994	6	0.01 – 0.2	No value	-	Not known
Polymeric MDI production	1995	6	0.01 – 0.2	No value	-	Not known
Polymeric MDI production	1996	6	0.01 – 0.2	No value	-	Not known
chemical industry	1996	30	-	0.015	-	Not known
Production	1996	15	0.002+0.021			Not known
Filling	1996	15	0.013+0.025			Not known
Pilot plant and Production	1979-1997	120	<LOD – 0.016	0.003		Not known
Polymerisation plants	1979-1997	40	<LOD – 0.015	0.0017		Not known
Laboratory	1979-1997	28	<LOD – 0.058	0.0045		Not known

Table 4.1 continued overleaf

Table 4.1 continued Workplace measurements from the chemical industry

Job category / activities	Year of measurement	Number of samples	Range of measurement data (mg/m3)	Median (mg/m3)	95% percentile	Techniques / sampling used
Production	1993-1998	44	0.002 – 0.052 [5]	0.007	0.014	Personal sampling, HP1050 Autosampler, HPLC
Maintenance	1993-1998	44	0.002 – 0.046 [5]	0.002	0.01	Personal sampling, HP1050 Autosampler, HPLC
Laboratory activities	1993-1998	22	0.002 – 0.02 [5]	0.0075	0.02	Personal sampling, HP1050 Autosampler, HPLC
Drums and Truck loading	1993-1998	43	0.002 – 0.16 [5]	0.008	0.053	Personal sampling, HP1050 Autosampler, HPLC
Prepolymer plant filling reaction vessel storage	1996		0.012 (n = 8) [6] 0.01 (n = 6) [6] 0.017 (n = 3) [6]			Static ? sampling: GMD Autostep 925 (paper tape) each sample in triplicate highest measurement is reported?
Prepolymer plant filling reaction vessel storage	1997		0.012 (n = 8) [6] 0.01 (n = 8) [6] 0.01 (n = 2) [6]			Static ? sampling: GMD Autostep 925 (paper tape) each sample in triplicate highest measurement is reported?
Drumming	1996	2	< 0.01			Personal sampling Using TLD-1, MDA Scientific, papertape method, calibration curve MDI-II
Product development	1996	2	< 0.01			Personal sampling Using TLD-1, MDA Scientific, papertape method, calibration curve MDI-II

1) 74% or more of all measurements were below the limit of detection
2) Limit of detection: 0.006 mg/m³ (no explanation is given for the lower value given in the table)
3) Limit of detection: 0.09 mg/m³
4) Limit of detection: 0.01 mg/m³
5) Limit of detection: 0.002 mg/m³
6) Llimit of detection: 0.01 mg/m³? (accuracy: 15%?)

The measurement results presented are assumed to be valid. The meaningfulness of the measurement results is limited, since it was not clear as to which tasks were included in the air sampling. In addition, information on the duration and frequency of exposure as well as information on the exposed persons is missing. Also sampling and analytical methods were poorly documented. Unless clarified otherwise, it is assumed that the samples were full-shift samples.

Confidential data from producers showed mostly exposure levels from below 0.0005 mg/m^3 to 0.058 mg/m^3, except for 2 production sites where peak exposure levels up to 0.2 mg/m^3 were measured. The meaningfulness of the latter peak exposure levels is limited by the poor documentation of the measurements. The exposure level of 0.058 mg/m^3 was measured in a laboratory.

No exposure data from production sites were found in a literature search.

For inhalation exposure to vapours of substances with low volatility (< 0.001 kPa) in a closed system without breaching, the EASE model estimates an exposure level of 0 – 1 mg/m^3 for a reasonable worst case [4].

According to information provided by industry, workers are said not to be exposed to the high process temperatures of 150–200°C in normal working conditions, but they are exposed to products at temperatures of up to 40–50°C. Therefore for further modelling, a temperature of 40°C has been used. In this case the EASE model predicts a worst case exposure, which is "negligible", regardless of pattern of use and pattern of control. However, it must be noted that the EASE model considers an exposure as negligible if the exposure is below 1 mg/m^3, which may still be too high for isocyanates.

Table 4.2 Worst case exposure levels calculated with EASE:

Work situation	Use pattern	Calculated exposure level
Production, closed system	Closed system without breaching	0 – 1 mg/m³
Production, closed system with breaching	Non-dispersive use with uncontrolled direct handling Exposure temperature: 40°C	0 – 1 mg/m³
Laboratory	Non-dispersive use with Local Exhaust Ventilation Exposure temperature: 40°C	0 – 1 mg/m³

However in situations where exposure is possible, exposure is normally controlled by the use of engineering controls, such as local exhaust ventilation. PPE is specified for all procedures and is determined by temperature of the operation. It is also estimated that duration of inhalation exposure due to specific activities is low (1-2 hours/day). Sampling takes ¼ hour/day for 350 days/year; filter replacement ½ hour/day for 24 days/year; equipment opening 2 hours/day for 60 days/year; vessel entry 8 hours/day for 10 days/year.

Laboratory work is normally done in fume cupboards for 8 hours/day.

[4] From the results given and the comments made below, it should appear that the EASE model is not at all appropriate for a compound such as MDI. Nevertheless, the EASE model has been used, but one should not put too much weight on the conclusions drawn from it.

As a reasonable worst case estimate for this scenario, an exposure level of 0.053 mg/m^3 will be used, since such a 95 percentile value has been reported by industry (even though it is not known, and actually unrealistic, that this value would be reached for an 8-hour exposure).

Short-term levels are expected to be about twice the reasonable worst case levels (expert judgement). As a short-term exposure estimate, 0.1 mg/m³ will be used.

For a typical exposure estimate, a median exposure level of 0.007 mg/m³ can be used, since such median values have been reported by industry. Nevertheless, we are aware of the fact that in most circumstances this median will never be reached.

Dermal exposure

Dermal exposure is theoretically possible during sampling, filter replacement, equipment opening for maintenance preparations, vessel entry and cleaning operations, e.g. hydroblasting, and in the laboratory.

No dermal exposure data are available, neither from the chemical industry, nor from a literature search.

For dermal exposure the EASE model estimates the following exposures for a reasonable worst case at a process temperature of 150–200°C (production in a closed system):

- closed system without breaching: very low

For incidental dermal exposure during production:

- non-dispersive use with direct handling and incidental contact: 0–0.1 mg/cm^2/day

For cleaning and maintenance during production, laboratory work:

- non-dispersive use with direct handling and extensive contact: 1-5 mg/cm^2/day

Table 4.3 Worst case exposure levels calculated with EASE

Work situation	Use pattern	Calculated exposure level
Production, closed system	Closed system without breaching	Very low
Production, closed system with breaching	Non-dispersive use with direct handling and incidental contact	0 – 0.1 mg/cm²/day 0 – 42 mg/day
Production, maintenance, cleaning Laboratory work	Non-dispersive use with direct handling and extensive contact	1 – 5 mg/cm²/day 130 – 650 mg/day

For incidental contact during production, it is assumed that only one hand is exposed, which corresponds to an exposed area of 420 cm^2. The dermal exposure is thus calculated as 420 · (0-0.1) which ranges from 0 to 42 mg/day. Consequently the "reasonable" worst case is estimated to be 42 mg/day.

For extensive contact during maintenance and cleaning activities during production and for laboratory work, an exposed area of 1,300 cm² (hands/parts of forearms) and a 10% dilution is assumed (according to TNO, The Netherlands). The dermal exposure is thus calculated as

1,300 · (1 – 5) · 0.1 which ranges from 130 – 650 mg/day. The "reasonable" worst case is estimated to be 650 mg/day.

It has to be noted that for dermal exposure, the EASE model defaults are considered excessively high as applied to the production of MDI but they have to be used in the absence of exposure data.

In a first attempt to make an estimation of the skin permeation, the SKINPERM model (version 9.01) was used. These results are presented in Annex 2 under 4.1.1.2.1.

As mathematical modelling is considered inappropriate for MDI, the available *in vivo* scientific data on dermal uptake are used for further calculations. According to Leibold et al. (1998), a dermal absorption of 1% can be assumed. Using a dermal absorption of 1% and the calculated dermal exposure of 650 mg/day, a dermal uptake of 0.01 · 650 = 6.5 mg/day is derived.

Because of the risk of irritation and sensitisation, extra measures are expected to be taken, including protective gloves. On the other hand, as mentioned earlier in this assessment, isocyanates and chlorinated solvents tend to affect rubber and most plastics and increase the risk of splitting the gloves. Gloves should be replaced as soon as there is any significant hardening and before protection time is exceeded.

Dermal exposure is highly dependent on the work practices, the use of PPE and the hygienic behaviour of the operators.

Combined exposure

For the calculation of the combined exposure the following assumptions are made:

- respiratory volume worker: 10 m³/day (TGD, 1996: default value)
- body weight worker: 70 kg (TGD, 1996: default value)
- absorption inhalation exposure: 100% (TGD, 1996: upper limit default value)
- absorption dermal exposure: 1% (Leibold et al., 1998)

The combined potential exposure is calculated with the worst case inhalation exposure of 0.053 mg/m³ (0.53 mg/day or 0.0076 mg/kg/day) combined with the worst case dermal exposure of 650 mg/day (9.2857 mg/kg/day), resulting in a combined exposure of 650.50 mg/day or 9.29 mg/kg/day.

Using the worst case inhalation exposure of 0.053 mg/m³ (0.53 mg/day or 0.0076 mg/kg/day), combined with the worst case skin uptake of 6.5 mg/day (0.0929 mg/kg/day), the total body burden would be 7.03 mg/day or 0.10 mg/kg /day.

However, the dermal route of exposure is believed to be prevented with appropriate protective equipment, which should be worn. The main concern remains for inhalation exposure.

4.1.1.2.2 Scenario 2: Downstream users, MDI as an intermediate in the industrial and skilled trade sectors

As already mentioned, MDI is mainly used in the production of rigid polyurethane foams. However, there are also many applications in the fields of Coatings, Adhesives, Sealants and Elastomers (CASE) such as paints, adhesives, weather resistant sealing materials and footwear. MDI is also used in the production of particle board and mould cores for the foundry industry.

In view of the diversity of possible applications, the frequency of handling per year may range from occasionally to daily, but at present it cannot be described in detail because of inadequate information on particular working activities within the various fields. It should be noted too that some types of exposures, most notably in the field of CASE, may involve small companies or workshops with little tradition of, or opportunities for, adequate work hygiene.

Inhalation exposure

Although MDI has a low vapour pressure, an aerosol of MDI can result in inhalatory exposure to MDI. This is the case during spraying operations; although no open spraying operations of MDI alone is known. Only reaction mixtures are sprayed. In hardeners for two-pack polyurethane systems, the MDI content is comprised between 1 and 10% if the product is solvent borne and between 40 and 65% if the product is solvent free (Ullmann Encyclopaedia of Industrial Chemistry 6th edition 1998, completed by data from paint manufacturers). Moreover, high exposure to isocyanates and amines potentially should occur when polyurethanes are thermally decomposed and more complex isocyanate compounds are formed (Skarping et al., 1995). This would be the case for pipe-layers welding polyurethane insulated pipes and for workers heating polyurethane glue with heat guns (ejected air as high as 500°C). It has to be noted that the use of personal protection equipment has been very limited among pipe-layers.[5] Foundry workers are also at risk of being exposed when using MDI based core materials. These materials may be decomposed by the heat of the melted metal, partly into low molecular isocyanates and partly into MDI.

A compilation of occupational exposure data from a wide range of applications and processes using MDI-based products was collected by industry and is presented in **Table 4.4**:

Table 4.4 Occupational exposure data collected by industry

	Application	Application details/remarks	Sampling	Level (mg/m³)	Number of samples
Rigid PU foam	lamination		static	LOD – 0.00004	23
	continuous lamination		static	LOD – 0.0167	21
	lamination		static	<0.001 – 0.0019	45
	refrigerators, freezers, boilers		static+pers.	LOD – 0.005	50
	boiler insulation		static	<0.001 - <0.02	20
	block foam		personal	<0.00002–0.00215	10
	roof panels for thermal insulation	dry thermal curing	static+pers.	>0.050	15
	one component foam filling with 1K-PU aerosol		static	0.002 – 0.003	2
	one component foam thermal insulation	steam curing	personal	0.00067-0.0047	3
			static+pers.	0.005 – 0.01	13
(Semi) Flexible PU foam	slabstock		personal	<0.00002–0.00156	6
	in-situ packaging foam		personal	<0.01 – 0.041	34

Table 4.4 continued overleaf

[5] Although the risk from exposure to decomposition products of polyurethane formally falls beyond the scope of this assessment, it is worth mentioning that MDI exposure can theoretically also occur in this context.

Table 4.4 continued Occupational exposure data collected by industry

	Application	Application details/remarks	Sampling	Level (mg/m3)	Number of samples
C.A.S.E.	spray floor coating		static+pers.	>0.050	15
	bridge decking; primer	pure in solvent	static+pers.	<0.030 – 0.119 1)	19
	bridge decking: membrane		static+pers.	<0.030 – 0.030	18
	spraying waterproofing material		static+pers.	<0.001 – 0.003	20
	spraying waterproofing material; primer		static	LOD – 0.008	46
	coatings		static	LOD	16
	hot melt roller coating with special design extraction	2% free MDI, appl. temp 125C	static	0.031	unknown
	oriented strand board	sprayed MDI	personal	0.000001 - 0.0543	48
	particle board	sprayed MDI	personal	<0.00003–0.04699	33
	particle board	sprayed MDI	static	LOD – >0.050 (9 meas. > 0.050)	140
	medium density fibreboard	sprayed MDI	personal	<0.00001 – 0.0128	35
	thermoplastic polyurethane / elastomer	sprayed MDI	personal	<0.00002–0.00835	50
	rubber crumb (old tyres) tyres	sprayed MDI	personal	<0.00026–0.00128	6
	window frames		static+pers.	LOD – 0.005	2)
			static+pers.	LOD – 0.005	8
MDI: blending and distribution		pure	personal	0.00155 – 0.0318	4
Paint producer, filling plant		MDI + hardener	static	LOD	unknown

1) The high values are measured at the point of application; spray head
2) 1 survey, number of samples unknown
LOD Limit of detection (approximately 0.00001 mg/m^3)
Static Fixed position monitors, which are used in a variety of places in progress
Personal The IOM sampler is worn by the operative throughout the period of work being monitored

It has been assured by industry that these data (obtained over the past 5 years) have been measured by today's state-of-the art methods, which capture total inhalable MDI, i.e. vapour and aerosol. However, quality control of these data is not generally available.

The values received were measured over a variety of exposure times (15 minutes – 8 hours), but they are represented here as 8 hours values expressed in mg/m^3, presenting the highest possible values, i.e. the worst case scenario. There is no data provided on the median value or the 95-percentile.

Unless otherwise stated in the application column of the table, MDI is always found in admixture, i.e. it is always mixed with a NCO reactive component (e.g. polyol) prior to dispensing.

Supplementary occupational exposure data during processing, performed at customer premises, gave comparable results.

Table 4.5 gives an overview to ISOPA's request for occupational exposure data at downstream users' sites. In total, 15 foam producers (1 company gave exposure data on both rigid and flexible foam production), 6 C.A.S.E. producers and 2 of unknown sectors replied. Two companies submitted only environmental related monitoring data. One company replied 'unable to give relevant data' with the reason: no production of MDI. Another company gave an extremely brief and unclear reply in Italian.

Table 4.5 Occupational exposure data from downstream users' sites collected by ISOPA

	Application	Application details/remarks	Sampling	Level (mg/m³)	Number of samples (year)
Rigid PU Foam 1	rebonded foam sampling method =? in 'Gefahrstoffverordnung'?	demoulding/transport of blocks	personal (2h)	<0.005	1 (1999)
2	lamination	operator at spray nozzle	personal	0.0003 - 0.031 (dosimeter + OSHA nr 47)	1 per year ('96-'98)
3		handling/ decanting drums (in cabinet with LEV)	static+pers. 36 minutes	<0.003 MDHS 25	2 or 4? (1994)
4	lamination	spray nozzle (or lay-down?) + LEV	personal (6h40)	< 0.00003 (<LOD) MDHS 25 (independent org.)	(1998)
5	cold cure moulding	trimming packaging	pers. 387' pers. 399'	< LOD < LOD	(1996) (2 yearly)
		demoulding trimming venting	personal (dur. ?) modif. OSHA n° 47 (HPLC fluoresc)	0.0002 0.0001 0.0003	(1999)
		demoulding, venting, trimming, packaging	static (dur. ?)	< LOD MDA, TLD1 SP Chemcasette	(1999) (6 -monthly)
6	cold cure moulding	(de)moulding	static (dur. ?)	< LOD MDA, TLD1	yearly
7	foam production (continuous PIR block production)	foamer saw operator superviser	personal (+/- 1h.)	0.0015 0.0022 0.0007	(1997)

Table 4.5 continued overleaf

Table 4.5 continued Occupational exposure data from downstream users' sites collected by ISOPA

	Application	Application details/remarks	Sampling	Level (mg/m3)	Number of samples (year)
		at saw (dur. 45') 2m after lay-down (dur. 11')	static	0.0024 <0.0005	(1997)
	(continuous VIP block production)	foamer saw operator superviser	personal (+/- 1h.)	0.0015 <0.0001 0.0017	(1997)
		at saw (dur. 16')	static	0.0013	(1997)
				MDHS 25/2; UV-electrochem. Detection data on climatic conditions	
8	block foam production	injection/mixing head	static (dur. 20') 1x	all measurements: <LOD (LOD= 0.007mg/m³)	(1996)
9	block foam production (chipfoam plant; no further details than here mentioned)	foaming channel	1x at 3 different points static (short term)	sure spot method (?) <0.045 0.03 < LOD < LOD < LOD	monthly January February March April May '99
(Semi) Flexible 1 PU foam	block foam	foaming machine	?	< LOD (routine checks for TDI)	?
2	laboratory: blending/foaming	in fume cupboard	static + personal (15 min.)	<0.003 -0.03 MDHS 25	4 (1997)
3	small moulding operation (prepolymer MDI)	(measurements in vicinity operator)	personal	<0.01 chemical tape	several times
4	two component in situ foam filling	dispensing equipment using pre-heated components	static?	<0.01 0.02 TLD-1 Chemcasette	30 1 ('91-'98)
5	thermoplastic PU	extrusion (6 works stations, no further details)	static (4h)	<0.001 (LOD) OSHA n°47	yearly?

Table 4.5 continued overleaf

Table 4.5 continued Occupational exposure data from downstream users' sites collected by ISOPA

	Application	Application details/remarks	Sampling	Level (mg/m3)	Number of samples (year)
(hard foaming?) 6	foaming process in automotive sector	moulding/foaming	personal (92', 1x)	(independent org.) cassette 0.00326	(1998)
7	flexible faced foam panels	laydown	pers. 5h33 pers. 4h10 static. 2h39 static 2h38	0.00009 0.00028 0.00028 0.01000	July '98
		bonding line	pers. 4h7	0.00004	
		chemical manipulation	pers. 5 hours 19	0.00003	
				MDHS25/2 and UV/electrochemical detection system	
C.A.S.E. 1	chipboard (in continuous)	blending	static + personal 4-8 hours.	<0.015 MDHS235/2	1 (1998)
2	adhesives - sealants	mixer loading + fitting operation	Personal (30-400min.)	0.003 (= max. of reported values)	5 (1/year: '94 to '98)
3	coating of wheels, rings,... (prepolymers TDI/MDI)	manually blending	personal 15', 3x	<0.001 (=LOD)	(1999)
	warm curing moulding	and moulding	15', 2x	<0.001 (=LOD)	
				OSHA meth. N°47 HPLC fluoresc.	
4	coating PU on conveyor belt	blending machine + LEV	personal (2x)	<0.0006	(1997)
		spraying in cabin + conditioning	static (2x)	<0.0006	(1997)
5	PU resins	production: pumping over chemical reaction	4 hours 1 hour	M.U.488 UNICHIM <0.001 5521NIOSH <0.001	every 6 months

LOD Limit of detection
Static Fixed position monitors, which are used in a variety of places in progress
Personal The IOM sampler is worn by the operative throughout the period of work being monitored
MDA MDA Scientific, Inc. "paper sampler"

In a recently published paper (Maddison P. 1998 – Huntsman Polyurethanes) air measurements from the wood panel industry were put together. Personal monitoring data ranged from none detected (N.D.) to 0.0470 mg/m³ for MDI (n=162). Area sample values ranged from N.D. to 0.27173 mg/m³ for MDI (n=336). No personal samples for MDI produced values >0.05 mg/m³. Approximately 2.5% of the area samples for MDI were >0.05 mg/m³. The majority of the area

samples that resulted in high MDI values were from sample positions or operations considered as "risk" areas, for example, above the press, opening of inspection hatches, over open weigh belts.

Additional information has been made available by the 'Berufsgenossenschaft der Chemischen Industrie (BG Chemie, pers. com., 1998)' who monitors diisocyanates concentrations at different production sites. So far 1,238 measurements have been recorded for MDI from which only 31 measurements were above the occupational exposure limit value (0.05 mg/m^3) and only 138 measurements above 0.0125 mg/m^3.

The results from the BIA-Report 4/95 Isocyanate (Hauptverband der gewerblichen Berufsgenossenschaften, 1995), show that the following fields of work generally fall far below the limit values: surface coating, gluing, processing synthetic foam, assembly, insulating and packing foams. A higher level of exposure was established for cavity insulation, mould foaming and block foaming, presses, casters and extruders for synthetics.

In the SUVA-report of Bereich Chemie (1992), Rossinelli reports, for the period 1989-1992, MDI concentrations of 0.009 up to 0.071 mg/m³ (3 personal samples) and 0.031 up to 0.440 mg/m³ (8 area samples). These measures were taken in a company using a two-component system on the basis of MDI for the lamination of the inside of castings. For this spraying application a pressure of 35 bars was used.

The Swedish government made also some information available on MDI exposure in the rubber industry for 1997 (Swedish National Board of Occupational Safety and Health, pers. com, 1998). The method of analysis used was the Swedish method for sampling with a bubbler impinger and MAMA (9-(N-methylaminomethyl) anthracene in toluene). These short-term measurements (± 15 minutes) were taken during the start up, the production and the checking of the production, stopping, rinsing, closing and cleaning. The MDI concentrations varied from <0.001 to <0.019 mg/m^3. The latter concentration was reached with a ventilation system not working properly. Stopping, rinsing, closing and cleaning gave concentrations up to 0.011 mg/m^3.

The Swedish National Board of Occupational Safety and Health in collaboration with the Labour Inspectorate are working in a monitoring project in Sweden (Swedish National Board of Occupational Safety and Health, pers. com., 1998). This 3 year project will be finished in 1999. In this project measurements have been made in workplaces using isocyanates. In 1999 the project is focussed on hot work in polyurethane. The results are still preliminary but would be published in the beginning of 2000. According to the Swedish National Board of Occupational Safety and Health, preliminary results from this ongoing monitoring project show exposure values just above 0.05 mg/m³ in two cases using spraying operations. In a foundry, values of 0.7 and 0.65 mg/m³ were measured when pouring melted metal on cores made from MDI. All these measurements were made with the recommended method mentioned previously in Section 4.1.1.1.

The exposure information received on MDI from the U.K. Health and Safety Executive is summarised in **Table 4.6** and **Table 4.7**.

Table 4.6 Occupational exposure data collected by the U.K.HSE

	Work task	Local Exhaust Ventilation	Sampling	Level (mg/m³)	Number of samples
Surface coating, including spray painting	mixing, weighing, operation of coating machines	with/without	personal	≤ 0.001	12
	coating operations	-	static	≤ 0.001 – 0.197	22
	spray painting	without	personal	≤ 0.001 – 0.004	5
	spray painting	without	static	≤ 0.001	2
Polyurethane production	Resin blending	without	personal	≤ 0.004	1
	moulding	without	personal	≤ 0.004 – 0.0125 1)	11
Foam production	mixing	with	personal	≤ 0.001	2
	spraying	with	personal	≤ 0.001 – 0.005	5
	injection moulding	with/without	personal	≤ 0.001	9
	demoulding	without	personal	≤ 0.002	12
	background measurements	-	static	≤ 0.001 – 0.007	36
Printing and laminating	operating laminating equipment	without	personal	≤ 0.001 – 0.005	12
	rolling	-	personal	≤ 0.005	6
	background measurements	-	static	≤ 0.001 – 0.005	-
Adhesives	manual application	without	personal	≤ 0.001	2
	spray application	-	personal	0.007	1
Foundry cores	making foundry cores	with	static and personal	≤ 0.001	5

1) Work was intermittent for results at the lower end of the range and continuous for the results at the higher end of the range. The top 4 results, 0.003, 0.004, 0.0075 and 0.0125 mg/m³ MDI were all obtained at the same factory, where compression type moulding appeared to be carried out.

Data generated from samples prior to 1994 is likely to have been obtained by drawing air through an impinger containing 1(2-methoxyphenyl) piperazine (1-2MP) solution with subsequent analysis by HPLC (MDHS 25). Following the revision of the above method by the UK HSE in August 1994, subsequent data may have been derived using either an impinger containing 1-2MP solution or a glass fibre filter impregnated with the same reagent or a combination of both (for aerosols) with subsequent analysis by HPLC (MDHS 25/2).

All operations measured were considered to be typical working conditions. The values received were measured over a variety of exposure times (15 minutes – 8 hours.), but they are represented as 8 hr values expressed in mg/m^3. Although the measurement results presented are assumed to be valid, the meaningfulness of these results is somewhat limited because they are poorly documented.

Table 4.7 Occupational exposure data collected by the U.K.HSE (additional data)

	Exposure level (mg/m³)
Spray surface coating	0.001 - 0.065
Polyurethane production	0.002 – 0.005 (measured total isocyanates)
Foam production – injection mould	0.001 – 0.004
Foam production – spraying	0.001 – 0.007
Printing and lamination	0.002 – 0.005
Adhesives	0.001 – 0.175
Foundry cores	0.001 – 0.006 (measured total isocyanates)

Some measurements were made for total isocyanates although MDI was the only diisocyanate in use.

The additional data in **Table 4.7** show that the HSE measured higher exposures for some applications. For coating applications (spray surface coating), a 15-minute exposure to MDI of 0.065 mg/m³ was measured. For spraying adhesives in plastics processing, a 53-minute exposure to MDI of 0.175 mg/m³ was measured.

The data from a literature search (Hagmar et al., 1993b; Erban, 1987; Woellner et al., 1997; Zammit-Tabona et al., 1983; Pham et al., 1978; Cvitanovic et al., 1989; Castillon, 2000) correspond to the aforementioned available data. Thus, exposure was usually lower than 0.05 mg/m³, but peaks up to 0.48 mg/m³ were occasionally measured. Crespo and Galán (1999) published data on personal worker exposures to MDI while dwellings and office buildings were being insulated with polyurethane foam. Personal samples of airborne MDI were taken at 17 construction sites run by different companies (specialist contractors). An impinger using a 1-(2-methoxyphenyl) piperazine toluene solution as absorbent was used to take personal samples for the sprayer and helper during indoor and outdoor applications (method MTA/MA-034/95), with 20-75 minutes per sample and a flow rate of 1 l/minute. The samples were analysed by HPLC. The analytical results showed that the levels of exposure were substantial, especially for the sprayer, with values of time-weighted concentrations of up to 0.077 mg/m³ of monomeric isocyanate for outdoor applications on the roof, and 0.400 mg/m³ of monomeric isocyanate for indoor applications, with 9 of 13 values being between 0.1 and 0.3 mg/m³. The helper's exposure was always lower. The highest individual sample measured indoor on the sprayer was 0.570 mg/m³.

A wide range of applications and processes using MDI-based products means a wide range of required **process temperatures**. Room temperature processing (moderately elevated temperatures of 50°C) is used for processing cast polyurethane elastomers. In the foam industry, process temperatures up to 165°C are required. In the rubber industry, fast vulcanising needs a temperature of 180°C and above. Cold cure moulding requires 25°C-45°C. However some (reaction injection) moulding systems (elastomers) require elevated temperatures up to 200°C-230°C. Overheating should be avoided, as significant polymer degradation may occur above 230°C.

The curing of aqueous two-pack PU coatings takes place either at room temperature or by stoving at around 130-140°C (Reed, 1997; Kahl et al., 1997).

The **production methods** include both closed, fully automatic processes and also partially open manual processes depending on the purpose of application. For on-site foaming, pouring and spraying, mostly with a hand-held, high-pressure spray gun (2-component, rigid foam), an approved respirator is needed. One-component frothed foams (OCF's) are used in the building

trade and are delivered as pressurised cans. Curing starts immediately and moves from the outside inwards. Therefore emission and hence potential exposure virtually ceases once the outer coat is cured. Occupational exposure can also occur when using MDI-based polyurethane glue. In the heating procedure infrared heaters or heat guns (ejected air as high as 500°C) are used.

Sweden has a warning when using the one-component frothed foam in the building trade. When using the can, aerosols can arise depending on the gas pressure in the can (Swedish National Board of Occupational Safety and Health, 1996).

Exposure is likely to be intermittent and may occur during sampling and analysis, during filter changing, during connecting and disconnecting pipelines, during loading and unloading road-tankers, during repair, maintenance and cleaning activities, during process upsets at systems or units with higher temperatures, during spraying applications, during on-site weighing, mixing and foaming or coating, during welding, gluing, during accidents like spillages, split hoses, leaking drums, etc. Individual suppliers and trade associations such as ISOPA have introduced standard industry guidelines for transport, storage, handling and use of diisocyanates. According to this source: "Management procedures embracing routine inspections and maintenance schedules including replacements for such things as hoses are required to be in place to ensure continuous high standards of condition of equipment. Such activities have reduced the potential for accidental exposures over the past 20 years to the point that incidents such as hose splitting are now very infrequent in a market place supporting many thousand product user companies."

Estimation of the inhalative exposure level performed in accordance with the EASE model produces the following results:

For inhalation exposure to vapours of substances with low volatility in a closed system without breaching, the EASE model estimates an exposure level of 0 – 1 mg/m^3 for a reasonable worst case at a process temperature of 230°C.

For inhalation exposure to vapours of substances with low volatility in a closed system with breaching, or for partially open manual processes (non-dispersive use with uncontrolled direct handling), the EASE model estimates an exposure level of 500 – 1,000 mg/m^3 for a reasonable worst case at a process temperature of 230°C. For non-dispersive use with Local Exhaust Ventilation (LEV), the model predicts an exposure level of 5 – 30 mg/m^3.

According to information provided by industry, workers are said not to be exposed to these high process temperatures in normal working conditions, but they are exposed to products at temperatures of up to 40–50°C. Therefore for further modelling, a temperature of 50°C has been used. In this case, the EASE model predicts a worst case exposure of 0 – 1 mg/m^3, regardless of pattern of use and pattern of control.

- non-dispersive use with uncontrolled direct handling: 0 – 1 mg/m^3
- non-dispersive use with LEV: 0 – 1 mg/m^3

For inhalation exposure to an aerosol (wide dispersive use with uncontrolled direct handling) the EASE model predicts an exposure level of >10,000 mg/m^3, regardless of process temperature.

This predicted value is clearly a substantial overestimation if one considers the exposure information for spray-painting provided by the U.K. Health and Safety Executive (UK-HSE) and reported to range between <0.001 and 0.004 mg/m^3 (see **Table 4.6**). It is important to recognise also that the EASE model assumes that the spray paint is 100% MDI, which is clearly not the composition of such paints, while the exposure data represent the actual analysed quantity of MDI that workers were potentially exposed to.

Table 4.8 Worst case exposure levels calculated with EASE:

Work situation	Use pattern	Calculated exposure level
-fully automatic processes with breaching -partially open manual processes	Non-dispersive use with uncontrolled direct handling Exposure temperature: 40°C	0 – 1 mg/m³
Open manual processes	Non-dispersive use with Local Exhaust Ventilation Exposure temperature: 40°C	0 – 1 mg/m³
Spray-painting	Wide dispersive use with uncontrolled direct handling	>10,000 mg/m³

However in situations where exposure is possible, exposure is normally controlled by the use of engineering controls, such as local exhaust ventilation. PPE is specified for all procedures and is determined by temperature of the operation.

As a reasonable worst case estimate for this scenario (downstream users, on the whole), an exposure level of 0.05 mg/m^3 will be used, since such exposure levels are measured in different industries, and since from all reported 1,238 measurements from the BG Chemie only 31 measurements are exceeding the national permissible workplace exposure level (0.05 mg/m³), thus indicating that the 95 percentile value is below this level.

Typical short-term levels are expected to be about twice the reasonable worst case levels (expert judgement). As a short-term exposure estimate, 0.1 mg/m³ will be used.

However, special attention has to be paid to occupational exposure of foam applicators on building sites. These are usually specialist contractors. According to the literature (Crespo and Galán, 1999) higher exposure levels of MDI exposure were measured on building sites during the process of insulating buildings with sprayed polyurethane foam. For sprayers, time-weighted exposure levels of up to 0.077 mg/m³ and 0.400 mg/m³ of monomeric isocyanate were reached during outdoor and indoor applications, respectively. The highest individual sample reached 0.570 mg/m³ monomeric isocyanate. Consequently, for specialist contractor foam applicators, the exposure level of 0.40 mg/m³ will be used as a worst case estimate. As a short-term exposure estimate, 0.57 mg/m³ will be used.

Dermal exposure

Dermal exposure is theoretically possible during sampling and analysis, during filter replacement, during loading and unloading road-tankers, during repair, equipment opening for maintenance and cleaning activities, during process upsets, during spraying applications, during on-site weighing, mixing and foaming or coating in the building industry, during welding, gluing, during accidental spills.

No dermal exposure data are, however, available, neither from the industry, nor from a literature search.

Again, the EASE model was used because of the lack of exposure data. For dermal exposure, the EASE model defaults are considered excessively high as applied to the second scenario.

For dermal exposure the EASE model estimates for a reasonable worst case at a process temperature of 230°C:

- closed system without breaching: very low

For dermal exposure at a product temperature of 50°C:

- non-dispersive use with direct handling and intermittent use: 0.1 – 1 mg/cm^2/day

For dermal exposure due to spraying

- wide dispersive use with direct handling and extensive use: 5 – 15 mg/cm^2/day

Table 4.9 Worst case exposure levels calculated with EASE:

Work situation	Use pattern	Calculated exposure level
Fully automatic processes	Closed system without breaching	Very low
-fully automatic processes with breaching -partially open manual processes -open manual process	Non-dispersive use with direct handling and intermittent use	0.1– 1 mg/cm²/day 130-1,300mg/day
Spray-painting	Wide dispersive use with direct handling and extensive use	5 – 15 mg/cm²/day 4,225 – 12,675 mg/day

It is assumed that both hands and parts of the forearms can be exposed, corresponding to an exposed area of 1,300 cm^2. For most working operations the dermal exposure is calculated as 1,300 · (0.1 to 1) which ranges from 130 to 1,300 mg/day. Consequently the reasonable worst case for almost all applications is estimated to be 1,300 mg/day.

For spray painting the dermal exposure is calculated with EASE as 1,300 · (5 to 15) which ranges from 6,500 to 19,500 mg/day or 6.5 to 19.5 g/day. It has to be noted that the workers are exposed to a product containing MDI and not to the pure substance. As reported by industry, MDI is never sprayed as a 100% pure substance: if the product is solvent free, the MDI content is at most 65%. Consequently the reasonable worst case calculated with EASE for spraying is 1,300 · 15 · 0.65 = 12,675 mg/day or 12.7 g/day. However, based on measured data for spray painting, gathered with the fluorescent tracer technique, TNO has concluded that the dermal exposure on skin (1300 cm² hands/part of forearms) is up to 5,350 mg/day of total paint (4.1 mg/cm²) (Lansink et al., 1998). Based on these data, the reasonable worst case for spray painting is estimated to be 5,350 · 0.65 (percentage of MDI) = 3,500 mg/day or 3.5 g/day. The latter figure has also been considered applicable as a reasonable worst case for specialist contractor foam applicators (for whom no specific dermal data are available).

Using a dermal absorption of 1% (Leibold et al., 1998) and the calculated dermal exposure of 1,300 mg/day (most working operations), a dermal uptake of 0.01 · 1,300 = 13 mg/day is derived for most working operations. For sprayers and specialist contractor foam applicators (dermal exposure 3,500 mg/day), a dermal uptake of 0.01 · 3,500 = 35 mg/day is calculated.

The results obtained from modelling (SKINPERM) have been placed, by way of information, in Annex 2 under Section 4.1.1.2.2.

Because of the risk of irritation and sensitisation, extra measures must be taken, including protective gloves. On the other hand, as mentioned earlier in this assessment, isocyanates and chlorinated solvents tend to affect rubber and most plastics and increase the risk of splitting the gloves. Gloves should be replaced as soon as there is any significant hardening and before the protection time is exceeded.

Dermal exposure is highly dependent on the work practices, the use of suitable PPE and the hygienic behaviour of the operators.

Combined exposure

For the calculation of the combined exposure the following assumptions are made:

- respiratory volume worker: 10 m³/day (TGD, 1996: default value)
- body weight worker: 70 kg (TGD, 1996: default value)
- absorption inhalation exposure: 100% (TGD, 1996: upper limit default value)
- absorption dermal exposure: 1% (Leibold et al., 1998)

The combined potential exposure for this scenario (downstream users, on the whole) is calculated with the worst case inhalation exposure of 0.05 mg/m³ (0.5 mg/day or 0.0071 mg/kg/day) combined with the worst case dermal exposure of 1,300 mg/day (18.5714 mg/kg/day), resulting in a combined exposure of 1,300.5 mg/day or 18.58 mg/kg/day.

Using the worst case inhalation exposure of 0.05 mg/m³ (0.5 mg/day or 0.0071 mg/kg/day), combined with the worst case skin uptake of 13 mg/day (0.1857 mg/kg/day), the total body burden would be 13.5 mg/day or 0.19 mg/kg /day.

The dermal route of exposure is believed to be prevented with appropriate protective equipment, which should be worn. The main concern remains for inhalation exposure. However, it has to be kept in mind that for workers on building sites the (dermal) route of exposure is believed not to be prevented completely by appropriate protective equipment although this equipment is recommended and should be worn.

For specialist contractor foam applicators, the combined potential exposure is calculated with the worst case inhalation exposure of 0.4 mg/m³ (4 mg/day or 0.0571 mg/kg/day) combined with the worst case dermal exposure of 3,500 mg/day (50 mg/kg/day), resulting in a combined exposure of 3,504 mg/day or 50.06 mg/kg/day.

Using the worst case inhalation exposure of 0.4 mg/m³ (4 mg/day or 0.0571 mg/kg/day), combined with the worst case skin uptake of 35 mg/day (0.5 mg/kg/day), the total body burden would be 39 mg/day or 0.56 mg/kg /day.

An overview of the conclusions of the occupational exposure assessment is given in **Table 4.10**.

Table 4.10 Summary on the occupational exposure assessment

Scenario	Exposure		Estimated inhalation exposure level (mg/m³)						Estimated skin exposure	
			Full shift (8 hour time weighed average)				Short-term			
	Duration (h/day)	Frequency (day/year)	Typical	Method	Reasonable Worst Case	Method	Level	Method	mg/day	mg/kg. day
1 Chemical industry: MDI production Prepolymer production	Continuous (8-hour-shifts) and batch-synthesis	Continuous	0.007	Meas.[1]	0.053 1	Meas.[2] EASE	0.1	Expert	650	9.29
2 Industrial and skilled trade sectors: Breached closed systems, partially open and manual processes Spraying Specialist contractor foam applicators			Not estimated		0.05 1 >10,000 0.40	Meas.[2] EASE EASE Literature	0.1 0.57	Expert Literature	1,300 3,500 3,500	18.57 50 50

Meas.[1] Data taken from industry measurements; median value
Meas.[2] Data taken from industry measurements; 95 percentile
EASE Calculated with the EASE model
Expert Expert judgement; short-term exposure estimate = 2x Reasonable Worst Case
Literature Data found in the literature

4.1.1.3 Consumer exposure

Sources of exposure

The Danish Product Register (1997) reports that more than 40 tonnes of MDI containing products are used in private households per year. No more information on the consumer products and application types are readily available. It is also not clear to which extent the reported amount concerns unreacted MDI or not.

Hobby and related activities

Upon the request of the rapporteur for information related to MDI use in consumer products, DETIC[6] (Belgian-Luxembourg's association of producers and retailers of cleaning and maintenance products, adhesives and related products, Brussels) judged the responses obtained via a questionnaire sent to its members, although scarce yet representative for the Belgian market. The responses were compiled in **Table 4.11**.

Table 4.11 Consumer product data obtained via DETIC (1999, 2000)

Identification of the consumer product	Content of MDI in the product		Results of simulation testing and exposure potential	
	Volume added (%)	Content of free MDI (%)	Time needed to empty 1 unit (min)	Exposure route
Spray (foam)	40	± 15 to 20	5	inhalation skin
Putty/filler in cartridge	5	± 0.5	10	skin
Liquid glue for wood	50	± 10 to 25	10	skin

All applications occur potentially and most likely in an indoor environment (remark: revision of the November 2000 mentioned information: deleted products were erroneously reported in the context of consumer usage and relate to exclusive industrial uses (DETIC[7]).

No data was made available about the total quantity of MDI formulated in consumer products.

[6] DETIC. Mrs. P. Halleux, General Secretary, personal communication on 2.12.99;
[7] DETIC corrigendum, Febr. 2001.

Table 4.12 Consumer product data obtained via a questionnaire sent by ISOPA, FEICA, and CEPE (2001)

Identification of the consumer product	Estimated content of free MDI (%) in the product	Potential exposure data
PU foam 1-C 2-C	 7-10% hardener: 100% [1]	 Application: OCF Consumer pack size: 300, 500, 750 ml Intermittent use for a short duration: 5-10 minutes/5 year
PU glues and putty 1-C 2-C	 10% hardener: 100% [1]	
Adhesives (flooring) 1-C 2-C	 < 0.1-35% 3-20%	 For laying a wood-parquet with a 1-C: 0.5 kg adhesive/m² parquet is needed. Exposure time: 8 hours/day
Paints	< 0.1% 35%	Application: spraying (liquid roof coating) Consumer pack size: 750 ml, 2.5 l Infrequent use: once/10 to 15 years Application: brush or roller (decorative) Consumer pack size: 5 l

1 For 2-Component products, the hardener contains 100% MDI in an isomer/homologue-mixture. 2-C putties can be already mixed and thus may have a lower content.

Spray foam or One Component Foam (OCF): MDI-based OCF is offered in the consumer market and to professional tradesmen for use as a filler used to fill in small gaps in buildings (e.g. around window frames, between floor boards etc). In this context, the word 'spraying' is not entirely appropriate. The OCF is supplied in pressurised cans and is applied through a pre-expansion tube (always part of the package). The product is released from the nozzle as a viscous foam, rather than as a sprayed aerosol. Curing starts immediately and moves from the outside inwards. Therefore emission and hence potential exposure virtually ceases once the outer coat is cured. However, Sweden has a warning when using the one-component frothed foam.

PU wood adhesives are used for waterproof bonding and on moist wood. Flooring adhesives are used for wood-parquet.

PU paint is used as a primer for liquid roof coating, with a long in-service life (10 to 15 years), and for decorative painting.

It is confirmed by industry that hot melt adhesives are currently offered to the D.I.Y. market.

According to industry, even 2-C products are offered to the D.I.Y. market. One company stated: "Moreover, other products normally offered only to the craftsmen can reach end-consumers by self-service at craftsmen retailers". However, it is very unlikely that the consumer's working conditions are ever appropriate for the use of 2-C products containing free MDI.

Moreover, a non-systematic search was performed by the rapporteur to find out about the various products that are available in do-it-yourself shops in Belgium in order to help carry out the scenarios below.

Patient exposure to MDI-based casts

A special case of "consumer" exposure relates to the situation of a patient to whom a cast is applied for a bone fracture. In a report from Bruynzeel et al. (1993), the presence of MDI has been described in medical products, such as cast material. Information from the manufacturer of the casting material revealed that when uncured, the fibreglass-reinforced polyurethane casts contain, apart from fibreglass and small amounts of additives, isocyanate-terminated prepolymer and 4,4'-MDI. According to the 'Instructions for Use' of MDI-based casting tapes, before the casting is applied to the patient's skin, the thermoplastic material should be preconditioned by immersing it in water (20 -27°C) and firmly squeezing 4 to 5 times under the surface to ensure complete penetration of water into the body of the bandage. After removal from the dip water, the bandage should be applied in the form of a spiral. The cast may be moulded to its final shape during the last 30 seconds of its setting cycle, after which time it may be windowed or trimmed with shears or a standard cast saw. According to a well documented unpublished report, isocyanate measurements (HSE method MDHS 25/2) were performed by industry during the application of several types of commercially available MDI-prepolymer impregnated synthetic casting tapes. All sample airborne isocyanate concentrations were well below the occupational exposure limits (<0.004 mg/m³ NCO for 15 minutes sampling, and <0.001 mg/m³ NCO for 60 minutes sampling) during the application of a full leg cast.

Although it is not standard practice, in reality to finish off the cast, a heat gun is sometimes used, reaching a local heating of 300°C (de Boer and van Efferen, 1999). During this stage, the consumer can be exposed to MDI vapours, especially if no proper ventilation system (e.g. local extractor) is installed.

Finished MDI-based polyurethane products

Theoretically exposure could occur to residual free MDI through contact with products in whose manufacture process MDI is used. The determination of residual extractable monomeric 4,4'-MDI in cold-cure moulded flexible foam has been reported by industry (Schupp and Hoffmann, 1999). The residual content of monomeric MDI in flexible polyurethane foam products should be in the order of a few parts per million (if at all). During a dynamic fatigue test, run over 135 minutes at 40°C and 50% relative humidity, there was no MDI detectable in the air of the closed chamber (detection limit 6 ng/m³). During a contact test, where filters containing derivatisation agent were contacted with the foam surface for 5 days at 22°C while compressed to 75% of the original foam-height, no MDI was extractable (detection limit 1 µg per filter, which is 1 µg/25 cm²). In summary, small amounts of residual MDI are present in the matrix but do not diffuse out of this environment. Since the small amounts of residual MDI would not be available at the surface of the foam, the residual MDI cannot get into contact with human skin or evaporate in the surrounding air to be inhaled. It was therefore concluded in that report that the detected residual amounts of MDI in cold cure moulded flexible foam do not provide the basis for adverse health effects.

In a position paper on the emissions from particle board bonded with polyurea produced with polymeric MDI, Einbrodt (1991) comes to the conclusion that polyurea bonded particle boards do not emit hazardous concentrations of MDI, even when they are used over large areas. There is no evidence for the diffusion of the decomposition product MDA from the particle board and no

documentation showing that this decomposition product even occurs in particle board that is manufactured and used properly. When subjected to brief thermal stress (up to one hour), particle board bonded with polyurea from MDI and containing no other chemicals (wood preservatives, coatings, etc.) emits no toxic decompositions (other than carbon monoxide resulting from the pyrolysis or combustion of the wood constituents), which results in a non-calculable risk. Strangely, no mention is made of the pyrolysis of N-containing products. The findings of Einbrodt (1991) are confirmed by an earlier report from the Fraunhofer Institute for Wood Research/Wilhelm Klauditz Institute in Brunswick, 1985.

Consumers could theoretically be exposed during the burning of MDI-containing products, however good waste management practices (e.g. in municipal solid waste incinerator) prevent harmful emissions to be formed.

Levels of exposure to MDI

The following data (if available) are used for the assessment of consumer exposure:

- exposure data and dermal absorption data.
- physico-chemical data (molecular weight, log Kow, vapour pressure at room temperature, water solubility)
- contact parameters
- concentration parameters (e.g. percentage of MDI in foam, adhesive)
- results from consumer models

With respect to the above mentioned indicated consumer uses of MDI and the availability of information especially about the concentration of MDI in the consumer products, 4 exposure scenarios are considered: 1° spray painting (liquid roof coating), 2° the use of 'spray foam' (OCF), 3° glueing and the use of putty/filler in cartridge, painting with brush, 4° the use of hot melt adhesives.

The consumer exposure has been assessed by a 'worst case approach', as craftsmen and 'handy people' in general like doing D.I.Y. jobs.

Initially, the CONSEXPO model, version 2.0c was used in a first attempt to estimate the consumer exposure to MDI. However, modelling with CONSEXPO and SKINPERM is considered inappropriate to MDI, both for the calculated "dose" in CONSEXPO and the inability to account for the reactivity of MDI. The results obtained by modelling have been placed, by way of information, in Annex 2 under Section 4.1.1.3.

As scientifically derived data are available for the assessment of dermal absorption of MDI, the TGD simple algorithms and the *in vivo* scientific data on dermal uptake (1% dermal absorption according to Leibold et al., 1998) are used to calculate the dermal exposure.

4.1.1.3.1 Scenario 1: Spray painting (liquid roof coating)

The exposure routes, from the use of MDI-containing paint in a spray application, are by inhalation and by skin contact.

The use of PU paint as a primer for liquid roof coating should be infrequent (in view of its long in-service life). Container sizes sold are 750 ml and 2.5 litres. These paints contain < 0.1% free MDI.

The systematic use of personal protective equipment by the consumer is unlikely.

Inhalation exposure

The paint currently available for consumers contains < 0.1% of free MDI. For this spray painting application outdoors, natural dilution by wind reduces the presence of contaminants in the work area. It is also assumed that this activity takes place once/10 to 15 years. As no exposure data are available and modelling is not appropriate for a compound such as MDI, the rapporteur is of the opinion that inhalation exposure will be very low, at least with the current application.

In conclusion, for Scenario 1 (spray painting: liquid roof coating), no reasonable worst case estimate is derived as inhalation exposure is assumed to be very low (negligible), at least with the current application.

Dermal exposure

As for the occupational exposure assessment (Scenario 2, downstream users, spraying applications), the dermal exposure assessment for this scenario is based on measured data for spray painting (Lansink et al., 1998). It is assumed that both hands and parts of the forearms can be exposed, corresponding to an exposed area of 1,300 cm². A content of free MDI of 0.1% was presumed for the use of the PU-paint.

Calculations according to Lansink et al., 1998: dermal exposure on skin (1,300 cm²) will be up to 5,350 mg/day of total paint (4.1 mg/cm²). Based on these data, the reasonable worst case for spray painting is estimated to be 5,350 · 0.001 (percentage of free MDI) = 5.35 mg/day.

Using a dermal absorption of 1% (Leibold et al., 1998) and the calculated dermal exposure of 5.35 mg/day (or 0.076 mg/kg bw/day), a dermal uptake of (0.01 · 5.35) / 70 = 0.0008 mg/kg bw/day is derived.

In conclusion, for Scenario 1 (spray painting: liquid roof coating), a dermal exposure level of 5.35 mg/day will be used as a reasonable worst case estimate, with a dermal uptake of 0.0008 mg/kg bw/day. These figures are used for further risk characterisation.

As modelling is considered inappropriate for MDI, results obtained from modelling (SKINPERM) have been placed in Annex 2 under Section 4.1.1.3., by way of information.

4.1.1.3.2 Scenario 2: The use of ‘spray foam’ (OCF)

The rapporteur is aware of the fact that most companies have made efforts to provide the consumer with the safest and advanced OCF systems, however other companies do not.

As less safe practices cannot be excluded, the exposure routes from the use of MDI-containing ‘spray foam’ (OCF) are by inhalation and by skin contact.

Consumer use of OCF should be intermittent and of short duration. Consumer pack sizes for ‘spray foam’ are 300, 500, 750 ml. OCF contains 10% free MDI.

The systematic use of personal protective equipment by the consumer is unlikely.

Inhalation exposure

Exposure data for the use of OCF were provided by industry. One company reports atmospheric monitoring during the use of OCF in an occupational environment:

Table 4.13 Atmospheric measurements during the use of OCF

Year of measurement: 1985			Year of measurement: 1986		
Sample [1]	NCO level ppm v/v	Sampling	Sample [2]	NCO level mg/m³	Sampling
1A	0.00049	Personal	1A	0.0061	Personal
1B	0.00032	Static	1B	0.0012	Static
1C	0.00027	Static	1C	0.001	Static
2A	0.00017	Personal	2D	0.0027	Personal
2B	0.00037	Static	2E	0.0015	Static
2C	0.00020	Static			

1 Samples taken during the use of OCF for sealing commercial vehicle cabins
2 Samples taken during the use of OCF for fixing window frames (1A, 1B, 1C) and the sealing of wooden joints on uneven surfaces (2D, 2E)

The atmosphere was sampled through glass sintered bubblers (static samples) or glass DACO type impinger (personal samples) following the HPLC Nitro reagent method. The measurements were quite well documented.

From another company the following data from two applications, containing two tests, were provided. The four results showed 'MDI not detected', with detection limits of <0.00038, <0.00056, <0.008 and <0.012 mg/m^3 as 4,4'-MDI.

An additional publication was made available (Methner et al., 2000) with data related to the use of OCF in the construction industry. This publication supports the earlier data. In 1999, limited personal air samples (collected and analysed in accordance with the appropriate NIOSH or OSHA sampling and analytical methods) were taken while flooring contractors installed vinyl flooring using a small amount of an isocyanate-containing glue (n=3) and while MDI-containing foams were applied using an aerosol canister to seal gaps in the foundation and around windows and doors (n=3). These few samples collected showed exposure data < 0.02 mg MDI/m³ (detection limit not reported and no further explanation given). According to the authors, additional sampling is needed to better characterise exposure despite the fact that the samples were below OELs.

Although, the aforementioned monitoring data reflect the situation in an occupational environment, we assume that these exposure data are also applicable for the use of OCF by the consumer. Using these aforementioned exposure data, a worst case exposure of 0.0061 mg/m³ can be derived.

In conclusion, for Scenario 2 (use of OCF), an exposure level of 0.0061 mg/m³ (measured during the use of OCF for fixing window frames) will be used as a reasonable worst case estimate. This is also the short-term inhalation exposure level (testing time was 5 minutes 6 seconds).

Dermal exposure

It is considered that for dermal exposure, the use of OCF can be assessed by the same approach as spray painting, because the application of the 'spray foam' often leads to a considerable amount of product reaching the skin (practical experience).

Like for the occupational exposure assessment (Scenario 2, downstream users, spraying applications), the dermal exposure assessment for this scenario is based on measured data for spray painting (Lansink et al., 1998). It is assumed that both hands and parts of the forearms can be exposed, corresponding to an exposed area of 1,300 cm². A content of free MDI of 10% was presumed for the use of OCF.

Calculations according to Lansink et al., 1998: dermal exposure on skin (1,300 cm²) will be up to 5,350 mg/day of total paint (4.1 mg/cm²). Based on these data, the reasonable worst case for the use of OCF is estimated to be 5,350 · 0.10 = 535 mg/day.

Using a dermal absorption of 1% (Leibold et al., 1998) and the calculated dermal exposure of 535 mg/day (or 7.643 mg/kg bw/day), a dermal uptake of (0.01 · 535)/70 = 0.0764 mg/kg bw/day is derived.

Because spray foam is applied in a viscous liquid form and, hence, will cause less splashes and spots than spray paint, it is likely that the real dermal exposure will be less. However, dermal exposure is highly dependent on the work practices, the use of PPE and the hygienic behaviour of the operator.

In conclusion, for Scenario 2 (use of OCF), a dermal exposure level of 535 mg/day will be used as a reasonable worst case estimate, with a dermal uptake of 0.0764 mg/kg bw/day. Although these figures may considerably overestimate the dermal exposure level and uptake, these very worst case estimates are used for further risk characterisation.

As modelling is considered inappropriate for MDI, results obtained from modelling (SKINPERM) has been placed in Annex 2 under Section 4.1.1.3., by way of information.

4.1.1.3.3 Scenario 3: Gluing, the use of putty/filler adhesives, painting with brush/roller

The most important exposure route, from the use of MDI containing glues, putty/filler adhesives and paints, is by direct skin contact.

It is assumed that inhalation exposure (e.g. evaporation from mixture) is negligible, because of the very low vapour pressure of MDI at room temperature.

One company claims that their adhesives contain a maximum of 0.1% MDI. However, other companies declare that some of their adhesives, placed on the consumer market, contain a maximum of 35% of free MDI, e.g. MDI-based adhesives for wood-parquet. MDI-based paints should contain a maximum of 35% of free MDI.

On the do-it-yourself market, cans of 250 ml or 250 g liquid wood glue, cartridges of 300-400 g, and cans of 5 l paint can be bought (practical market information).

According to industry, 0.5 kg adhesive (containing 35% free MDI)/m² should be used for laying a wood-parquet. For a room of 12 m², a quantity of 6 kg adhesive has to be used; this is an equivalent to 2.1 kg free MDI. A total exposure time of 8 hours/day is assumed.

As mathematical modelling is considered inappropriate to MDI, both for the calculated "dose" in CONSEXPO and the inability to account for the reactivity of MDI, the TGD simple algorithms[8] and the *in vivo* scientific data on dermal uptake are used to calculate the dermal exposure estimation:

- Amount of undiluted product used: $q = 6 \cdot 10^6$ mg
- Volume of undiluted product used: $V_p = 6 \cdot 10^3$ cm³
- Weight fraction of substance in product: $w_f = 0.35$
- Dilution factor: D = 1
- Thickness of layer of product in contact with skin: $T_{der} = 0.01$ cm (default)
- Surface area of exposed skin: $S_{der} = 840$ cm²
- Body weight: 70 kg
- Dermal absorption (Leibold et al., 1998): 1%
- Average concentration of substance in product: $C_{der} = (q \ . \ w_f)/(V_p \ . \ D)$ (mg/cm³) = 350 mg/cm³
- Amount of substance on skin: $A_{der} = C_{der} \cdot T_{der} \cdot S_{der}$ (mg) = 2,940 mg
- Dermal uptake: $2{,}940 \cdot 0.01 = 29.4$ mg/day or 0.42 mg/kg bw/day

Based on these data, the reasonable worst case for dermal exposure for laying parquet is estimated to be 2,940 mg/day or 42 mg/kg bw/day, with a dermal uptake 0.42 mg/kg bw/day. Laying parquet 4 times/year, for 40 years results in dermal exposure of 168 mg/kg bw/year, with a yearly dermal uptake of 1.68 mg/kg bw/year.

Although the rapporteur is aware of the probable overestimation of dermal exposure, by using the TGD simple algorithms, these figures are used for further risk characterisation.

During normal use, some incidental skin exposure may occur due to lack of skin protection. However, it is unlikely that the total surface of the hands will be covered during normal working conditions.

Results, obtained by modelling using CONSEXPO and SKINPERM, have been placed in Annex 2 under Section 4.1.1.3., by way of information.

4.1.1.3.4 Scenario 4: Hot melt adhesives

MDI-containing hot melt adhesives are available on the DIY market and in the retail trade (with heat gun included). This has been confirmed by industry and exposure data has been submitted.

The traditional melt temperature of a hot melt adhesive is 140°C-190°C. The cooling and hardening of the hot glue would take 60 to 90 seconds, after which the glue is stable and joints are connected firmly. However, the full curing time takes 1 to 5 days. Hot melt adhesives contain a maximum of 2% free MDI.

The systematic use of personal protective equipment by the consumer will be unlikely.

[8] Note that TGD simple algorithms give a very worst case exposure estimate

The consumer can be exposed to MDI using MDI-containing hot melt adhesives by direct skin contact and by inhalation of MDI vapours during heating.

Inhalation exposure (exposure by inhalation of MDI vapours during heating)

Exposure data, concerning the use of hot melt adhesives by consumers, has been submitted by one company in 2001. Their well-conducted experimental study reports exposures of up to 0.025 mg/m³ during the use of a hot melt adhesive with a specific heating-gun that was specially designed to minimise exposure of the operator to the glue. These exposure values were well documented and reached under worst case conditions. Consequently, an exposure level of 0.025 mg/m³ will be used as a reasonable worst case estimate for this scenario. This is also the short-term inhalation exposure level (testing time was 16-23 minutes).

Dermal exposure

As mathematical modelling is considered inappropriate to MDI, the dermal exposure estimation is calculated with the TGD simple algorithms[9] and the *in vivo* scientific data:

- Amount of undiluted product used: q = 65,000 mg
- Volume of undiluted product used: V_p = 65 cm³
- Weight fraction of substance in product: w_f = 0.02
- Dilution factor: D = 1
- Thickness of layer of product in contact with skin: T_{der} = 0.01 cm (default)
- Surface area of exposed skin: S_{der} = 840 cm²
- Bodyweight: 70 kg
- Dermal absorption (Leibold et al., 1998): 1%
- Average concentration of substance in product: $C_{der} = (q \cdot w_f) / (V_p \cdot D)$ (mg/cm³) = 20 mg/cm³
- Amount of substance on skin: $A_{der} = C_{der} \cdot T_{der} \cdot S_{der}$ (mg) = 168 mg
- Dermal uptake: 168 · 0.01 = 1.68 mg/day or 0.024 mg/kg bw · day

Based on these data, the reasonable worst case for dermal exposure for using hot melt adhesives is estimated to be 168 mg/day or 2.4 mg/kg bw/day, with a dermal uptake 0.024 mg/kg bw/day.

Although the rapporteur is aware of the probable overestimation of dermal exposure, by using the TGD simple algorithms (e.g. using a surface area of exposed skin being 840 cm² - both hands), these figures are used for further risk characterisation.

During normal use, some incidental skin exposure may occur due to lack of skin protection. However, it is unlikely that the total surface of the hands will be covered during normal working conditions – using one tenth (arbitrarily defined) of the surface area might be more realistic for calculating the dermal exposure. However, given the high temperature of the glue, skin burns are not inconceivable, thus resulting in a substantially elevated dermal uptake[10].

[9] Note that TGD simple algorithms give a very worst case exposure estimate

[10] Response to comments of the CSTEE adopted during the 41st plenary meeting of 8 January 2004.

By way of information, the results obtained by modelling using CONSEXPO and SKINPERM, have been placed in Annex 2 under Section 4.1.1.3.

4.1.1.4 Indirect exposure via the environment

The EUSES model has been used to calculate the MDI human daily dose through food, air and drinking water; data used to run the model were described in Section 3.1.

The main results are gathered in **Table 4.14**.

In this table, daily doses linked with releases in the local environment are expressed as a range of intake when considering all use patterns of MDI (figures given are minima and maxima).

Table 4.14 Estimated human daily intake of MDI from environment.

	Regional	Local
Daily dose through air (mg/kg.day)	$4.41\ 10^{-8}$	$1.74\ 10^{-7}$ – $5.04\ 10^{-6}$
Daily dose through drinking water (mg/kg.day)	$1.95\ 10^{-8}$	$1.95\ 10^{-8}$ – $1.41\ 10^{-7}$
Daily dose through fish (mg/kg.day)	$2.99\ 10^{-6}$	$2.99\ 10^{-6}$
Daily dose through leaf crop (mg/kg.day)	$8.78\ 10^{-8}$	$3.47\ 10^{-7}$ – $1\ 10^{-5}$
Daily dose through root crop (mg/kg.day)	$6.35\ 10^{-7}$	$8.11\ 10^{-7}$ – $7.31\ 10^{-6}$
Daily dose through meat (mg/kg.day)	$1.46\ 10^{-9}$	$5.23\ 10^{-9}$ – $1.47\ 10^{-7}$
Daily dose through milk (mg/kg.day)	$8.62\ 10^{-10}$	$3.09\ 10^{-9}$ – $8.66\ 10^{-8}$
Total daily intake for humans (mg/kg.day)	$3.78\ 10^{-6}$	$4.\ 35\ 10^{-6}$ – $2.57\ 10^{-5}$

At the regional level, intake from fish are the most important (79%); on the local scale, doses from fish and vegetable consumption and from the air are the most important sources of MDI intake by humans from the environment. The results were received by EUSES modelling.

However, given the reactivity of MDI with water and the information available on the absence of accumulation of MDI in organisms (see Section 3.1.4); it is very unlikely that MDI becomes available in the food chain.

4.1.1.5 Combined exposure

On the basis of the exposure estimates given in Section 4.1.1.2, 4.1.1.3 and 4.1.1.4 respectively, humans can be exposed to MDI as a result of combined exposure. The figures from Section 4.1.1.4 show that indirect exposure via the environment can be considered to be negligible for the calculation of the combined exposure.

Dietemann-Molard et al. (1991) reported on a 38-year-old man complaining of an immediate asthmatic reaction while insulating a window at home with a MDI-containing polyurethane foam. Eight years before the study, the man had experienced bronchospasm after burning polyurethane packs at work. After changing jobs, respiratory symptoms disappeared. After this acute clinical manifestation, the patient admitted having slight breathlessness two or three times when painting cars with isocyanate-containing paints.

Sommer et al. (2000) report on a case of occupational asthma caused by MDI casts in a 35-year old female nurse. The nurse worked in an emergency room, applying synthetic casts containing

MDI 0-3 times daily. Just before the last serious asthma attack, the nurse's husband had used insulation foam containing MDI.

As a reasonable worst-case (WC), someone who works in the industrial and skilled trade sector, and is exposed at home doing some 'D.I.Y.' (e.g. use of OCF) will receive a maximum dose of MDI which can be quantified as is shown in **Table 4.15**.

Table 4.15 Calculated exposure data concerning combined exposure

Exposure pathway	Acute exposure	Lifetime exposure[1]
Inhalative	Workplace[2] WC 0.05 mg/m³ Consumer[3] WC 0.0061 mg/m³	Workplace[2] WC 68.57 mg/kg bw Consumer[3] WC 0.14 mg/kg bw
Dermal	Workplace[4] WC exposure 1,300 mg/day, WC uptake 13 mg/day Consumer[5] WC exposure 535 mg/day WC uptake 5.35 mg/day	Workplace[4] WC exposure 178.3 g/kg bw, WC uptake 1783 mg/kg bw Consumer[5] WC exposure 1.2 g/kg bw, WC uptake 12 mg/kg bw

1 For the calculation of lifetime doses following parameters were used: 70 kg bw (adult), 1.25 m³/hour inhaled air for a consumer doing D.I.Y.-jobs for working 8h/day, 4 days/y for 40 years (consumer), 10 m³/8 hour workshift inhaled air, 5 days/week 48 weeks/y and 40 years working period (worker), dermal absorption 1%.
2 Worst case inhalation exposure for the occupational Scenarios 1 and 2 is 0.05 mg/m³.
3 Worst case inhalation exposure for consumer Scenario 2 (use of OCF) is 0.0061 mg/m³.
4 Worst case dermal exposure for almost all applications in the occupational Scenario 2 (industrial and skilled trade sector) is 1,300 mg/day.
5 Worst case dermal exposure for consumer Scenario 2 (use of OCF) is 535 mg/day.

4.1.2 Effects assessment: Hazard identification and Dose (concentration)-response (effect) assessment

4.1.2.1 Toxico-kinetics, metabolism and distribution

4.1.2.1.1 Studies in animals

Oral

There are no data available.

Dermal

In a study by Vock and Lutz (1997), female Wistar rats were treated topically with monomeric [^{14}C]4,4'-MDI in dried acetone on the clipped back, onto an area of about 3 · 3 cm of the resting hair growth, without occlusion. The animals were killed 24 hours or 48 hours after topical application. Faecal excretion of radioactivity amounted to 20% of the administered radioactivity within 24 hours. Urinary excretion was below 1%. About 10% of the radioactivity was retained at the site of application. Epidermal nuclear protein exhibited very high specific radioactivity but ^{32}P-postlabelling analysis did not reveal isocyanate-DNA adducts. The nuclear protein radioactivity in the liver, lung and kidney was much lower than in the epidermis. DNA radioactivity in the liver was at the limit of detection. Conversion to the units of the Covalent Binding Index, CBI = (µmol adduct/mol DNA nucleotide per mmol chemical administered/kg body weight) (Lutz, 1979, in: Vock and Lutz, 1997) resulted in a value of <0.1. According to the authors' conclusions, the presence of 2% MDA in the application solution could have contributed about 0.03 CBI-units to the measured values. In comparison with genotoxic carcinogens, the upper bound value is indicative of a very weak maximum possible systemic genotoxic potency of topically administered MDI.

In a draft report by Leibold et al. (1998), the absorption, distribution and excretion of radioactivity was studied in groups of 4 male Wistar rats following a single dermal and intradermal administration of ^{14}C-4,4'-MDI (4,4'-Methylenebis[ring-U-14C]-phenylisocyanate) at nominal dose levels of 4.0 and 0.4 mg/cm² for dermal (in dried acetone) and 0.4 mg/animal for intradermal administration (in corn oil). These dose levels corresponded to nominal dose levels of 40 and 4.0 mg/animal for dermal administration. In the experiments with dermal administration, animals were exposed for 8 hours and sacrificed 8, 24 or 120 hours after the beginning of exposure. For the dermal administration, the clipped area was first washed with acetone and a silicone ring was glued to the skin. After administration of the substance with a syringe, a nylon mesh gauze was then glued to the surface of the silicone ring and a porous bandage used to encircle the trunk of the animal. Generally, the largest proportion of radioactivity was recovered from the application site (low dose: up to 55.6%; high dose: up to 32.2%) and dressing (low dose: up to 50%; high dose: up to 69.1%). Dermal absorption was very limited and rather similar at both dose levels. About 0.9% of the radioactivity applied were absorbed at most. At the early time-points (8 and 24 hours), the absorbed radioactivity was excreted via urine and faeces in similar amounts. After 120 hours, excretion via faeces was predominant with the rate of excretion being relatively constant over this time period. After intradermal administration of ^{14}C-4,4'-MDI, the total amount of radioactivity absorbed amounted

to about 26% of the radioactivity applied. The absorbed radioactivity was excreted mainly via the faeces with the rate of excretion being relatively constant over this time period.

The studies by Vock and Lutz (1997) and by Leibold et al. (1998) show contradictory results. Neither of the two studies appear to be unreliable. The possibility, that contamination from grooming could explain the results in the study of Vock and Lutz, was evoked and judged by the authors not to provide an explanation for the findings. In the report of Leibold et al. (1998), the site of administration was occluded with a silicone ring, a nylon mesh gauze and a porous bandage, whereas in the study of Vock and Lutz (1997), the site of administration remained unoccluded. In the report of Leibold et al. (1998), the animals were only exposed for 8 hours. The only plausible explanation for the contradictory results we can find is that there might have been a competitive extraction of the ^{14}C-MDI to the dressing or other material used to occlude the application site in the study by Leibold et al. (1998), since up to 69.1% of the radioactivity was recovered from the dressing. From the study of Vock and Lutz, it must be concluded that absorption through the skin is not negligible.

The *in vitro* absorption from various doses of ^{14}C-4,4'-MDI in dried acetone (dermal doses of 0.04mg/cm² and 4.0 mg/cm²; unoccluded) through guinea pig, rat and human skin was studied by Clowes, 1997. In all skin types, the majority of the applied MDI equivalent was found to be associated with the skin (58 – 91% of dose) following decontamination of the skin surface by flushing with 3% Teepol in water. No radioactivity was detected to have been absorbed through human skin from any of the applications during a 54-hour continuous exposure. From the various doses applied to rat and guinea pig, absorbed radioactivity was detected in small amounts from only three of the applications and the absorption rate, in terms of MDI equivalent, was slow. This *in vitro* study, confirms the results obtained in the Leibold et al. (1998) report, in which absorption through the skin was minimal.

Inhalation

In the pharmacokinetic study of Lab. d'Etudes (1977) ^{14}C-MDI (monomeric 4,4'-MDI) dissolved in benzene was administered, head only, for 15 minutes to 12 Sprague-Dawley male rats by inhalation of an aerosol having a maximum droplet size of 5 microns. Only the solvent and part of the MDI were converted to aerosol, and it was impossible to calculate the MDI concentration in the inhalation chamber. The rats were killed at times from 15 minutes to 96 hours after administration. The ^{14}C-contents of blood, urine, faeces, and exhaled air were determined for variable numbers of animals after various time intervals. The ^{14}C-content of bile was measured for one animal.

Residual radioactivity was determined in most cases, and whole-body autoradiographs were taken in two cases (after 15 minutes and 24 hours). The most salient results were said to be:

- that faecal elimination of MDI and its metabolites was greater than urinary elimination.
- that after 4 days 70% of the absorbed dose was eliminated.
- that bile secretion during the first 46.5 hours was 5% of the dose received.
- that ^{14}C-MDI was fairly uniformly distributed throughout the organism, with a predominance for the lungs, muscle, kidneys and the digestive tract.
- that histological tests on the lung fragments showed congestion of the capillaries, desquamation and destruction of bronchial epithelium, and constriction of bronchi up to obstruction.

In a recent study (Kennedy and Brown, 1998) the biochemical and histoautoradiographic characterisation of the distribution of radioactivity following exposure to ^{14}C-MDI (monomeric 4,4'-MDI; purity of 98.9%) has been reported. Acute four hour exposures of groups of 4 male, Fischer 344 rats, via head-only inhalation, to three separate concentrations were performed. At 0.052 and 0.36 mg/m³ MDI concentrations, the isocyanate was ring labelled with ^{14}C, while in the 6 mg/m³ MDI concentration the exposure was performed with unlabelled MDI. Aerosol concentrations were stable throughout all exposures as monitored by two independent HPLC methods. Mean aerosol particle diameters were equivalent between experiments at 1.18 µm. All tissues examined showed detectable quantities of radioactivity, with the airways, gastrointestinal system and blood having the highest levels. The level of radioactivity was directly related to the exposure concentration and decreased gradually during the post-exposure period. A minimum plateau of radioactivity appeared to be reached at 120 hours post-exposure. 95-100% of the plasma radioactivity was recovered in the retentive fractions (>10 kDa). SDS PAGE analysis of the plasma fraction greater than 10 kDa showed a single, labelled component of 70 kDa, which points to biomolecular conjugates. It appeared that conjugation of MDI with proteins was the predominant reaction and that the free amine or other low molecular weight adducts or metabolites were not the predominant, *in vivo* reaction products under the conditions tested.

To complement the biochemical distributions, histoautographic analysis of airway sections from ^{14}C-exposed rats showed predominantly surface labelling but no significant airway alteration. Even at the highest MDI aerosol concentration (6 mg/m³), no changes in airway morphology were observed when compared to the unexposed control group immediate after exposure.

A number of studies (Sepai et al., 1995b; Sabbioni et al., 2000) have addressed the issue of the possible occurrence of adducts of MDI to haemoglobin (see below). The occurrence of such adducts is of potential relevance in the paradigm that covalently bound adducts of chemicals to haemoglobin (or other plasma proteins) may be indicative of the occurrence of adducts with DNA, as has been demonstrated with genotoxic agents such as ethylene oxide, propylene oxide, and aflatoxin. Moreover, such binding to proteins may be of relevance for the process of sensitisation. Consequently, the presence of protein adducts may also be of use for biological monitoring, since the amount of adducts would be indicative for the integrated exposure during the lifespan of the studied protein.

In the case of MDI-derived protein adducts, it is important not only to identify such adducts, but also to understand the mechanisms and pathways of their formation. In particular, it is important to establish whether and to what extent the adducts are produced directly, i.e. by a chemical reaction between the -N=C=O group(s) of MDI and functional groups on the protein, or indirectly after the biotransformation of MDI to MDA, and the subsequent CYP-dependent oxidation of -NH_2 groups to hydroxylamines (-NHOH). Isocyanate-specific adducts will consist of carbamoylated (R-NH-C(=O)-) aminoacids;

- in the case of adduct formation with the N-terminal NH_2 of valine, a urea is formed:
  R-N=C=O + H_2N-valine → R-NH-C(=O)-NH-val (which by rearrangement may lead to the formation of a hydantoin); hydrolysis of these adducts does not yield the corresponding amines
- in the case of adduct formation with cysteine (-SH), a thiocarbamate is formed:
  R-N=C=O + HS-cysteine → R-NH-C(=O)-S-cys

Arylamine-specific adducts will consist of sulfinamides in the case of adduct formation with cysteine:

R-NH_2 → R-NHOH → R-N=O + HS-cysteine → R-NH-S(=O)-cys,

which is easily hydrolysed (acid or base) to the amine ($R\text{-}NH_2$).

Two studies (Sepai et al., 1995b; Sabbioni et al., 2000) have been published regarding this issue. Both were conducted by the same researchers using the same material, i.e. a chronic inhalation study in rats (Hoymann et al., 1995), but they are not so easy to reconcile because the second study does not adequately confront its results with those of the first study. The first study concluded that significant amounts of arylamine-specific adducts were found in urine, whereas the second study found that isocyanate-specific adducts were at least 12 times more abundant than the arylamine-specific adducts. However, this does not invalidate the findings by Sepai et al. (1995b) that MDA-derived adducts are found after exposure to MDI. The two studies are reported in some more detail below.

Sepai et al. (1995b) investigated the occurrence of haemoglobin adducts and urine metabolites of 4,4'-MDA after exposure of rats to monomeric 4,4'-MDI starting from the long-term experiment (Hoymann et al., 1995), designed to determine the carcinogenic and toxic effects of MDI. In this study, female Wistar rats were exposed chronically for 3 and 12 months, to 0.0, 0.26, 0.70 and 2.06 mg MDI/m^3 as aerosol (17 hours/day, 5 days/week). Haemoglobin adducts and urine metabolites of MDI were determined at different doses in order to develop methods to biomonitor workers exposed to MDI and to assess a risk resulting from such exposure. Haemoglobin adducts and urine metabolites of MDA were found in all rats. MDA and N'-acetyl-4,4'-methylenedianiline (AcMDA) were quantified by GC-MS after derivatisation with heptafluorobutyric anhydride. The dose-response relationships for haemoglobin adducts and urine metabolites were non-linear over this dose range. In urine, free AcMDA and MDA were found after base extraction. The amount of MDA present in urine and to a lesser extent the AcMDA found in urine correlated well with the corresponding amount determined as haemoglobin adducts for all dose groups. In order to release MDA from possible conjugates of MDA and AcMDA, urine was treated under strong acidic conditions. Following this procedure higher MDA levels were found than the sum of MDA and AcMDA from mild base hydrolysis. Similar results were obtained with the rats exposed for 3 and 12 months, indicating that a steady state had been reached by 3 months. The haemoglobin adducts and urine metabolites are most probably not products formed by atmospheric hydrolysis of the diisocyanate to the amine, as MDA was not detected by the air monitors. Sepai et al. (1995b) assumed that MDI is first hydrolysed to MDA which is further oxidised to N-hydroxyarylamine and subsequently to the nitroso compound in the erythrocytes. The nitroso derivative of MDA and/or AcMDA would yield the sulphinamide adducts, which are acid and base labile. The authors concluded that it is unlikely that the adducts detected result from the reaction of the isocyanate with the amino acids of the haemoglobin, since acetylated MDA was found which proves that the isocyanate group was hydrolysed to the amine.

To distinguish between typical arylamine and isocyanate adducts, the same research group developed procedures for identifying the isocyanate-specific adduct with the N-terminal valine of haemoglobin (Sabbioni et al., 2000). The globin samples were prepared from the blood samples taken in the already mentioned long-term experiment (Hoymann et al., 1995 in Sepai et al., 1995b). For the quantification of haemoglobin adducts, N^{1-}[4 - (isocyanatobenzyl) - phenyl]acetamide (AcMDI) was reacted with the tripeptide valyl-glycyl-glycine and with valine yielding N - [4 - (4 - acetylaminobenzyl)phenyl]carbamoylvalyl-glycyl-glycine and N - [4 – 4 - acetylaminobenzyl) - phenyl] carbamoylvaline, respectively. N - [4 - [4 - (acetylamino - 3,5 - dideuteriobenzyl)-2,6-dideuteriophenyl]carbamoyl]valine was synthesised from valine, as was N^{1-}[4-(4-isocyanato-3,5-dideuteriobenzyl)-2,6-dideuteriophenyl]acetamide, for use as an internal standard. These adducts were cleaved in 2M HCl to yield the corresponding hydantoins, 3-[4-(4-aminobenzyl)phenyl]-5-isopropyl-1,3-imidazoline-2,4-dione (MDA-Val-Hyd) and 3-[4-(4-

amino-3,5-dideuteriobenzyl)-2,6-dideuteriophenyl]-5-isopropyl-1,3-imidazoline-2,4-dione, respectively. In the globin of the MDI exposed rats, MDA-Val-Hyd could be found in a dose-dependent manner. The adduct was identified by HPLC/MS/MS and quantified by GC/MS after derivatisation with heptafluorobutyric anhydride. The amount of MDA-Val-Hyd found after acid hydrolysis of globin at 100°C was about 12 times larger than the sum of N-acetyl-4,4'-methylenedianiline (AcMDA) and MDA obtained from mild base hydrolysis of haemoglobin (results of the Sepai et al., 1995b study). It was concluded that the MDA-Val-Hyd was an isocyanate-specific adduct and that the MDA and AcMDA released after mild base hydrolysis resulted most likely from a sulfinamide adduct which is a typical adduct of arylamines. According to these results, the authors concluded that higher amounts of isocyanate adducts than arylamine adducts should be expected in workers exposed to isocyanates.

The publication by Day et al. (1997) on TDI suggests that a different mechanism of haemoglobin or protein adduct formation could be anticipated. The first step might be the formation of a labile adduct between MDI and GSH. In a subsequent step the isocyanate is transferred to a more stable adduct with larger proteins. This mechanism seems to be more straightforward than the sequence of reactions via MDA and the nitroso compound suggested by Sepai et al. (1995b). This GSH transfer mechanism does not require the assumption that free MDA must occur as an intermediate metabolite. However, the fact that the isocyanate-specific adducts may result from the solvolysis of isocyanates bound to glutathione and then transported to sites distant from the lung (Day et al., 1997) does not influence the findings of the studies of Sepai et al. (1995b) and Sabbioni et al. (2000).

A full inhalation metabolism/toxicokinetics/distribution study on monomeric 4,4'-MDI has been performed by Gledhill (2001a) and published by Gledhill et al. (2005). The routes and rates of excretion and the tissue distribution of radioactivity in the male rat (6 groups of four Wistar-derived rats) following a 6 hour inhalation exposure (condensation aerosol, head-only) to ^{14}C-MDI at a nominal concentration of 2 mg/m³ was investigated according to OECD guideline 417. Immediately after the exposure, one group was killed by a lethal injection to determine the received dose. These rats were decapitated and the skin was separately removed from the head and the body. The head, skin from the head, carcass and pelt were analysed separately. The radioactivity in each of these tissues was summed for each animal to determine the received dose. Four groups were killed at intervals up to 168 hours after exposure and selected tissues taken for analysis in order to investigate tissue distribution of absorbed material. Urine and faeces were also collected from these rats. Rats in the fifth group were surgically fitted with a bile duct cannula prior to exposure and urine, bile and faeces were collected until 48 hours after dosing, when these rats were terminated. The radioactivity in excreta, bile and tissues was measured. In a separate exposure experiment, 4 male rats were exposed to a similar ^{14}C-MDI atmosphere for 6 hours before urine, faeces and exhaled CO_2 were collected for up to 36 hours.

The received mean total dose of radioactivity was 33.72 kBq per animal, being equivalent to 0.078 mg MDI equivalents per rat. Particle size analyses (MMAD 1.39 µm, GSD 2.01) and the amounts of radioactivity found in the lungs (approximately 13% of the dose) demonstrated that the ^{14}C-MDI atmospheres were highly respirable to the rats. Inhalation exposure of rats to ^{14}C-MDI resulted in the rats receiving a combined inhalation/oral dose as radioactivity deposited on the head and pelt during the exposure was ingested during grooming. During the 168 hour post-exposure period approximately 5% and 79% of the dose were excreted in urine and faeces, respectively. Over a 48 hour interval after exposure, bile duct cannulated rats excreted approximately 12% of the dose in urine, 14% in bile and 34% in faeces. In conclusion, most of the systemically available dose was excreted via bile with a small proportion excreted in urine. There was no radioactivity present in exhaled CO_2. Radioactivity was widely distributed, with

the respiratory and excretory organs containing the highest concentrations of radioactivity. These declined to low levels of residues in all tissues analysed after 168 hours. More in detail, immediately after the exposure period the highest proportions of radioactivity were found in the residual carcass and gastrointestinal content (accounting for 37% and 32% of the dose respectively). The lungs, gastrointestinal tract, liver and respiratory nasal tissue accounted for 13%, 4%, 3% and 1% of the dose respectively. All other tissues measured contained less than 1% of the received dose. The highest concentration of radioactivity (67 µg equiv./g) was present in the respiratory nasal tissue. The lungs and trachea contained 7 and 3 µg equiv./g respectively, and the olfactory nasal tissue and thyroid each contained approximately 1.0 µg equiv./g respectively. The concentration of radioactivity in each of the other tissues analysed was 0.5 µg equiv./g or less. At 8 hours after the end of exposure the gastrointestinal contents contained the highest proportion of radioactivity, accounting for 48% of the dose. The residual carcass contained 34% of the dose and the lungs 10%. Respiratory nasal tissue and the liver contained 4% and 3% of the dose respectively, and the gastrointestinal tract and stomach contents each contained 2% of the dose. Again, the respiratory nasal tissue contained the highest concentration of radioactivity, 394 µg equiv./g. The lung, trachea and olfactory nasal tissue contained the next greatest concentrations: 5 µg equiv./g in the lungs and 1 µg equiv./g in each of the tracea and olfactory nasal tissue. Other tissues contained radioactivity at 0.5 µg equiv./g or less. By 24 hours after the end of exposure the highest proportion of radioactivity was present in the residual carcass and the gastrointestinal contents (19% and 13% of the dose respectively). Lungs and liver were the only other tissues containing appreciable amounts of radioactivity, 6% and 2% of the dose respectively. All other tissues contained radioactivity at or below 1% of the dose. Respiratory nasal tissue, lungs and trachea contained radioactivity at concentrations of 17.3 and 1 µg equiv./g respectively. The concentration of radioactivity in the olfactory nasal tissue was 0.3 µg equiv./g and in the adrenals 0.2 µg equiv./g. All other tissues contained radioactivity concentrations of 0.2 µg equiv./g or less. At 168 hours after exposure, the lungs contained 4% of the administered dose and the gastrointestinal contents contained 1% of the dose. The residual carcass contained 5% of the dose. All other tissues contained less than 1% of the dose. Respiratory nasal tissue, thyroid and the lungs contained the highest concentrations of radioactivity, 3 µg equiv./g, 3 µg equiv./g, and 2 µg equiv./g respectively. The concentration of radioactivity in the adrenals was 0.3 µg equiv./g. All other tissues contained less than 0.2 µg equiv./g.

According to the author, the tissue distribution of radioactivity at different time points after exposure, when considered with the excretion data, imply that the systemic doses were mainly due to absorption of radioactive material present in the gastrointestinal tract after ingestion, with pulmonary absorption of radioactivity deposited in the lungs accounting for only a minor, hardly quantifiable portion of the systemic dose. Immediately at the end of the exposure period 32% of the received dose was present in the gastrointestinal tract, which could be explained by ingestion of radioactivity deposited on the head and the respiratory tract during exposure. The residual carcass analysed immediately at the end of the exposure period contained 37% of the received dose, with 36% of this present on the skin and 1% present in the true residual carcass (without skin). At 168 hours after dosing, the radioactivity in the residual carcass had decreased to 5% of the received dose. This decrease in radioactivity was accompanied by a concomitant increase in the cumulative excretion of radioactivity in the faeces. It was found reasonable by the author, to conclude therefore, that radioactivity present on the skin was ingested during grooming. This conclusion was supported by the excretion, from the bile duct cannuled rats, of 34% of the received dose in faeces. If the exposure to radioactivity was exclusively via the inhalation route, the radioactivity in faeces from these animals would have been negligible.

In order to better understand the toxicokinetic behaviour of MDI after inhalation exposure, biological samples from these experiments (urine, faeces, bile) were investigated (Gledhill, 2001b and Gledhill et al., 2005). Urine, faeces and bile were collected for the identification of metabolites at 6 (in urine and bile only), 12, 24, 36 and 48 hours (and for intact animals at 24 hourly intervals until 7 days after the end of exposure). Metabolites present in bile and excreta were identified by LC/MS and/or by co-chromatography with reference standards and quantified.

There was no MDA detected in any of the samples analysed for this study. With the exception of 1 minor metabolite, all low molecular weight metabolites present in urine and bile were identified or characterised as follows:

Metabolite I: N,N'-diacetyl-4,4'-diaminobenzhydrol

This was the major urinary metabolite in both intact and bile duct cannulated rats (1% and 6% of the dose respectively). It was also present in bile (1% of the dose).

Metabolite II: N,N'diacetyl-4,4'-diaminophenylmethane

This metabolite was present in urine of intact and cannulated rats (0.5% and 4% of the dose respectively) and was present as the major metabolite in bile (4% of the dose).

Metabolite III: N-acetyl-4,4'diaminophenylmethane

Metabolite IV: N,N'-diacetyl-4,4'-diaminobenzophenone

Metabolites III and IV were minor urinary metabolites (< 0.5% of the dose) and were not present in bile.

None of these specified low molecular weight metabolites were found in faeces.

The solvent extract of faeces and the major radioactive component in bile (9% of the dose) was thought to consist of mixed molecular weight polyurea oligomers derived from MDI. The implication of these results, made by the author, is that a proportion of the MDI dose (10%) is converted to these metabolites via intermediary formation of an amine group which is rapidly acetylated. Both formation and acetylation are most likely to occur within specific cells or compartments. It is not possible from the current data to elucidate whether this stage involves:

- MDA, although none was detected
- bound-MDA, i.e. as a bound intermediate on an enzyme involved in the formation of the metabolites,
- an amine group resulting from reversion of the expected MDI-glutathione conjugates as proposed below:

  Lung: MDI + 2GSH $\rightarrow$ GSH-MDI-SHG

  Tissues: GSH-MDI-SHG $\rightarrow$ GSH-MD-NCO $\rightarrow$ GSH-MD-NH_2 $\rightarrow$ GSH-MD-$NHCOCH_3$

Reversal of the second glutathione link would lead to the formation of Metabolite III, with subsequent metabolism but without free MDA having been formed at any stage.

Currently, there are no data to confirm any of the above possibilities, all of which are feasible biochemically, and hence it is not yet possible to describe quantitatively their respective roles in MDI biotransformation.

The III is in the process of elucidating further the steps on the biological transport and transformation of MDI. Glutathione conjugates are considered to play an important role here by reference to TDI, methyl isocyanate, isothiocyanates and other compounds that are converted *in vivo* to isocyanates, all of which conjugate reversibly to glutathione (Baillie and Kassahun, 1994). Fully authenticated samples of mono- and bis-glutathione conjugates of MDI have been synthesised and degradation in simple *in vitro* systems is being investigated (Reisser et al., 2001). Results to date show: biologically relevant half-lives for these conjugates (approximately 1 hour for bis adduct, 8 hours for mono adduct); polyurea formation from the mono-glutathione adduct; no detectable free amine arising from either conjugate upon degradation.

Further studies using biologically relevant *in vitro* systems are about to commence.

In a study by Bartsch et al. (1996), pregnant Wistar rats were exposed, whole body, once for 6 hours on day 19 post coitum (pc) to 20 mg/m^3 monomeric 4,4'-MDI as aerosol, immediately followed by the collection of maternal blood, amniotic fluid, foetus, placenta. This study is described very briefly and no more relevant data are available regarding the set up of the experiment. In this study transplacental transition of MDI or degradation products was shown. MDA analysis was done after acid hydrolysis; therefore it is not possible to differentiate between MDI and MDA. The MDA analysis, after acid hydrolysis, demonstrated that the highest MDI or degradation product levels were measured in the maternal blood, followed by the placenta, foetus and amniotic fluid. The levels in the placenta were 66.4%, in the foetus 42.4% and in the amniotic fluid 13.6% of the maternal blood levels.

Pauluhn (2002b) compared the relative sensitivity of markers of exposure and effects in the lung of female Wistar rats exposed (directed-flow nose-only) to pMDI aerosol. Rats were repeatedly exposed to 12.9 mg pMDI/m³ (6 hours/day, 5 days/week for 14 days; exposure from days 0-17 followed by a post-exposure period to day 35). Markers of exposure, the amines that relate to both 4,4'-MDI (4,4'-MDA and 4,4'-AcMDA) and 3-oligomeric MDI (3-core MDA), were determined in bronchoalveolar lavage (BAL), blood (haemoglobin, plasma proteins), and urine on days 1, 4, 11, 18, 21, 28 and 35. Markers of effects were determined at the same time points and focused on changes in BAL constituents. In BAL, a maximum increase of total protein occurred following the first exposure and levelled off subsequently whilst BAL cell-related endpoints increased in a time-dependent manner. The kinetics of formation and elimination of adducts differed appreciably from one dosimeter to another. Whilst haemoglobin adducts were integrated by the cumulative exposures, the incremental yield of adduct formation appeared to be dependent on pulmonary as well as yet unknown erythrocyte-related factors. Plasma protein adducts attained a plateau after 1 week of exposure. MDI-related metabolite levels in urine did not show any time-dependent changes during the entire exposure period and declined rapidly during the post-exposure period. The kinetics of the fractional loading and clearance of pulmonary and extrapulmonary dosimeters did not parallel each other, nor was there a clear correlation with the markers of effects. The author concluded that biomonitoring is a powerful tool for the comparative dosimetry of well-defined exposure regimens. However, for irritant agents demonstrating portal-of-entry effects, markers related to 'total body burden' may not necessarily predict the absence or presence of local responses occurring within the target organ.

As biomonitoring can integrate past exposures that result from diverse routes, Pauluhn and Lewalter (2002) analysed the yields of markers of pMDI following a single inhalation or single dermal exposure of female Wistar rats (n = 6) and extended a previous study with repeated exposure to pMDI aerosol (for a more detailed description of the experimental design: Pauluhn, 2002b). This analysis included the hydrolysis products of 4,4'-MDI and its 3-oligomeric congener (3-core MDI). As exposure-site specific reactions may result in hydrolysis and/or conjugation/denaturation of scavenge or tissue proteins, also equimolar doses of the amine

analogs, 4,4'-MDA and 3-core MDA were studied. For the inhalation exposure, rats were acutely exposed, directed-flow nose-only, for a duration of 6 hours to 3.7 mg pMDI/m³ and 2.7 mg MDA/m³, respectively. Furthermore, C · t products of ~1,200 mg pMDI/m³ · hour were examined, ranging from 3 hours · 6.2 mg/m³, 1.5 hours · 12.7 mg/m³, 45 minutes · 25.1 mg/m³, and 23 minutes · 58.1 mg/m³. For pMDI and MDA the MMAD were in the range of 1.5-1.7 µm (GSD 1.5 – 2.1) and 1.0 µm (GSD 1.9), respectively. Additional groups of rats received equimolar doses of pMDI and MDA by epicutaneous exposure (first method with spacer; second method with Elizabethan collars), i.e. 100 mg pMDI/kg bw, equivalent to 50 mg 4,4'-MDI/kg bw and 34 mg 3-core MDI/kg bw or 79 mg MDA-mixture/kg bw, equivalent to 46 mg 4,4'-MDA/kg bw and 33 mg 3-core MDA/kg bw. Markers of exposure were determined in hydrolysed urine and blood (haemoglobin adducts). The sampling of urine started shortly after cessation of exposure overnight. Haemoglobin adducts were subjected to alkaline hydrolysis, urine was hydrolysed using concentrated hydrochloric acid. The biomarkers determined (AcMDA/4,4'-MDA ratios in haemoglobin and 4,4'-MDA/3-oligomeric MDA in urine) suggest that the kind and yield of biomarkers are dependent on the route of exposure and differ markedly for MDI and MDA. For both routes, the yield of the urinary marker (related to 4,4'-MDI) was approximately 2-orders of magnitude higher than that of haemoglobin. For each marker the yield obtained by the dermal route was 1-order of magnitude lower when compared to inhalation. In contrast, following dermal and inhalation exposure to MDA, the efficiency to produce haemoglobin adducts was about 2-orders of magnitude higher when compared to equimolar exposures to the isocyanate. Based on 4,4'-MDA in urine 1-order of magnitude higher concentrations were detected relative to pMDI. The isocyanate appears to undergo chemical reactions specific to the site of first contact (e.g. formation of adducts, conjugates and/or polyureas) suggesting that these markers of 'total body burden' can neither predict the local dose at that site nor do they provide any means to identify the route receiving the most critical dose. According to the authors, it appears that the formation of biomarkers is governed by reactions requiring an intact isocyanate group rather than hydrolysis to the amine. In addition to inhalation exposure, massive dermal exposures to pMDI appear to be necessary to increase markedly the respective biomarkers. According to the authors, it can be speculated that trace amounts of materials of low volatility tend to remain on surfaces, becoming a potential exposure source, i.e. diamines subject to dermal exposure may dominate the outcome of analysis and over predict significantly putative exposures to isocyanates using currently available procedures.

Other routes

In a preliminary study of Lab. D'Etudes (1976), monomeric 4,4'-MDI, ^{14}C-labelled at the methylene carbon atom, was administered to a small number of fasting male and female Sprague-Dawley rats by a single intramuscular injection into the right hind paw. Blood samples were taken at regular time intervals. Urine and faecal samples were collected during 12 or 24-hour periods up to 120 hours after administration. The exhaled $^{14}CO_2$ was measured as a function of time. At the end of the experiment the animals were killed, homogenised and the radioactivity measured. From the blood samples it was estimated that the half-life of diffusion from the muscle was 12 hours, and the biological half-life for elimination of ^{14}C-MDI and its metabolites was 78 hours. Faecal elimination of the ^{14}C was greater than the urinary elimination. Exhaled ^{14}C, not as $^{14}CO_2$, appeared after 2 days. The excreta recovery balance was < 25%, with the remaining ^{14}C being found in the carcasses.

4.1.2.1.2 Studies in humans

There are few data available on the toxicokinetics of MDI in humans. The only relevant findings relate to the use of biomarkers in the biomonitoring of workers exposed to 4,4'-MDI.

Haemoglobin (Hb) adducts and urine metabolites were analysed by Schütze et al., 1995. Workers exposed exclusively to MDI were studied. Exposure levels, as monitored using personal air samplers, were below the detection limit of 3 μg/m^3, with the exception of 3 individuals. In 10 of the MDI workers (17 in total), hydrolysable Hb adducts of MDA (57-219 fmol/g Hb) were found. Except for 4 subjects, the presence of MDA and AcMDA was detected in all urine samples after base treatment. Following acid hydrolysis of the urine, higher levels of MDA (0.7-10 nmol/l) were found than the sum of free MDA and AcMDA. According to the present data, it was possible to detect exposure to MDI in a greater number of individuals by analysing urinary metabolites than by measuring Hb adducts or air monitoring.

In a review article Skarping et al. (1995) described the presence of MDA[11] in plasma and urine in a worker, who repaired a MDI conveyer belt by blowing hot air (350°-600°C) onto its surface and imprinting on it with a metal roller. In an attempt to reproduce the conditions during the incident, the concentration of MDI in the breathing zone was measured and found to be 15-μg/m^3. The illness was suggestive of an MDI-associated illness, compatible with both immediate hypersensitivity and a complement-mediated immune-complex reaction. The patient's blood and urine samples were analysed for the presence of 4,4'-MDA using acid hydrolysis. The first urine sample was obtained 22 hours and the last sample 114 hours after start of exposure. The urine concentrations of MDA were corrected for creatinine. The half-time of excretion was 70-80 hours. The first serum sample was obtained 19 hours and the last sample 1,967 days after the start of exposure. The half-time was 21 days, which suggests the presence of plasma protein adducts in the exposed worker. It is possible that blood analyses better reflect the past exposure to MDI: at least in the reported patient such interpretation is suggested.

In a short communication, Sepai et al. (1995a) discussed albumin adducts, haemoglobin adducts and urinary metabolites in workers exposed to 4,4'-MDI. They obtained biological samples from a group of 20 workers exposed to MDI vapour during the manufacture of polyurethane products. The air levels were monitored and compared with the plasma protein adduct, Hb adduct and urinary metabolite levels. The blood and urine samples were analysed for the presence of adducts and metabolites using GC-MS methods. Urinary base-extractable metabolites were found above control levels in 15 of the 20 workers and ranged from 0.035 to 0.83 pmol MDA/ml. The level of the acetylated metabolite N'acetyl-4,4'-methylenedianiline (AcMDA) ranged from 0.13 to 7.61 pmol/ml. The amount of MDA released after acid hydrolysis was on average 6.5 times higher than the amount of free MDA and AcMDA present in urine. MDA was detected as a haemoglobin (Hb) adduct in all of the 20 subjects. The level ranged from 70 to 710 fmol/g Hb. In one individual the Hb adduct of AcMDA was detected. As plasma albumin conjugates of MDI can cause the onset of respiratory disorders in both human and animal models, the presence of plasma protein adducts was investigated. The plasma MDA levels ranged from 0.25 to 5.4 pmol/ml. Up to 120 fmol/ml were found to be covalently bound to albumin.

Dalene et al. (1996) investigated whether exposure of pipe-layers to thermal degradation products of MDI could be assessed by analysing 4,4'-MDA in acid hydrolysed plasma and urine,

[11] It should be noted that with the current state of the art, the term 'MDI metabolite' instead of 'MDA' would be more appropriate (see the analysis method used at that time and the results of the recent studies by Gledhill et al. 2001b and 2005).

and whether the genotype for N-acetylation affected these biomarker levels. Blood and urine samples were drawn from 30 pipe-layers who had been welding polyurethane insulated pipes during the preceding 3 months. MDA in plasma was detected in 18 of the 30 pipe-layers. Their plasma concentration of MDA varied from 0.05 to 8.48 μg/l. There was a significant negative correlation between time since last welding of polyurethane insulated pipes and plasma MDA (r_s = 0.50, P = 0.005). There was a significant positive correlation between the estimated number of welded polyurethane insulated pipes during the preceding 3 months and plasma MDA (r_s = 0.68, P = 0.001). No significant association between genotype of N-acetylation and plasma MDA was observed in a multiple regression analysis when adjustment was made for the estimated cumulative exposure thermal degradation products of MDI. MDA in urine was detected in only four of the 30 pipe-layers. Plasma MDA, after acid hydrolysis, but not urine MDA, therefore seems to be a useful biomarker of long-term exposure to MDI. The individual N-acetylation capacity did not affect the plasma levels of MDA.

While Sepai et al. (1995a) and others have shown MDA, MDA adduct etc., in human urine and blood, the accuracy of the levels measured can be questioned due to some of the analytical methology employed, i.e. the use of pmol levels deuterated standards to aid in detection of fmol levels in the biological samples. However, the consistency of findings linked to similar findings in animals suggests that MDI may undergo similar metabolic conversion in humans, although it is not yet elucidated how much of the MDA found results from the metabolic conversion of MDI or from other processes, possibly related to the method of analysis. For a better understanding of the uptake of MDI in the airways and its toxicokinetics, controlled exposure studies of humans could be performed. Because bronchial provocation testing with MDI (or other isocyanates) may be required for diagnostic purposes in cases of suspected occupational asthma (Vandenplas and Malo, 1997), these “experimental” exposures could be used to gain more insight into the toxicokinetics of MDI in humans, provided all current ethical guidelines are adhered to.

4.1.2.1.3 Summary

In general, the information on the toxicokinetics of MDI is limited. Although some studies on the metabolism of MDI are available, it must be considered that the fate and biotransformation of MDI has still to be fully elucidated. No information is available on the toxicokinetics of MDI following oral exposure in animals.

With respect to dermal exposure, contradictory results have been obtained, with none of the studies giving ground for rejection so that it must be concluded that absorption of MDI through the skin must not be neglected. Consequently, a dermal uptake of 1% (Leibold et al., 1998) is used to calculate the body burden in the dermal exposure assessment.

With respect to inhalation exposure, there are good reliable data regarding distribution/excretion in experimental animals. One study indicates that MDI is uniformly distributed throughout the organism, with a predominance for the lungs, muscle, kidneys and the digestive tract. The faecal elimination of MDI and its metabolites is greater than the urinary elimination. These results were confirmed by the preliminary results from the full inhalation metabolism/toxicokinetics/distribution study performed by Gledhill (2001a) and published by Gledhill et al. (2005). In this study, approximately 5% of the dose was excreted in urine and 79% in faeces of intact animals. Bile duct cannulated animals excreted approximately 12% of the dose in urine, 14% in bile and 34% of the dose in faeces. Radioactivity was widely distributed with the respiratory and excretory organs containing the highest concentrations of radioactivity. In some other biomonitoring studies haemoglobin adducts and urine metabolites of MDI were determined. Haemoglobin adducts and urine metabolites of MDI were found in all animals. In

another study the transplacental transition of MDI or degradation products (MDX) after MDI inhalation were detected. The MDA analysis, after acid hydrolysis, demonstrated that the highest MDI metabolite levels were measured in the maternal blood, followed by the placenta, foetus and amniotic fluid.

From the data generated to date, it is possible to state that MDA is not a significant metabolite of inhaled MDI in the rat. The vigorous acid hydrolysis used in biomonitoring studies will convert MDI and MDI conjugates to MDA. Only carefully controlled metabolism studies will provide the definitive answer to any metabolic conversion of MDI to MDA or whether alternative possibilities, such as involvement of GSH as discussed, predominate. The results of the inhalation metabolism/toxicokinetics/distribution study performed by Gledhill (2001b) and published by Gledhill et al. (2005) indicate that a proportion of the MDI dose is converted to metabolites via the intermediary formation of an amine group which is rapidly acetylated. However, it is not possible from the current data to elucidate the steps in the biological transport and transformation of MDI. Glutathione conjugates are considered to play an important role. Further studies using biologically relevant *in vitro* systems are about to commence.

The few data available on the toxicokinetics of MDI in humans result from monitoring studies. A report indicates that it is possible to detect exposure to MDI in a greater number of individuals by analysing urinary metabolites than by measuring Hb adducts or air monitoring. In another report the half-life of MDA in hydrolysed urine was determined to be 70-80 hours, the half-life in serum was 21 days. Similar results obtained in other studies indicate that plasma metabolites seem to be a useful biomarker of long-term exposure to MDI.

Nevertheless for a better understanding of the uptake of MDI in the airways and its toxicokinetics, controlled exposure studies of humans should be performed. Because bronchial provocation testing with MDI (or other isocyanates) may be required for diagnostic purposes in cases of suspected occupational asthma (Vandenplas and Malo, 1997), these “experimental” exposures could be used to gain more insight into the toxicokinetics of MDI in humans, provided all current ethical guidelines are adhered to.

Dermal

Table 4.16 Overview of studies on toxicokinetics and distribution: dermal route

Substance	Species	Method	Endpoint	Reference	Reliability[1]
^{14}C-4,4’-MDI	Rat	Toxicokinetics and distribution after a single dermal (in dried acetone) and intradermal (in corn oil) administration	Dermal absorption was limited: 0.9% of the ^{14}C applied was absorbed. Absorbed ^{14}C was excreted mainly via the faeces with the rate of excretion being relatively constant.	Leibold et al., 1998	3
^{14}C-4,4’-MDI in dried acetone	Rat	Toxicokinetics and distribution after a single topical admin.	Faecal excretion of ^{14}C: ~ 20% within 24 hours. Urinary excretion < 1%. 10% of ^{14}C retained at application site;	Vock and Lutz, 1997	2

Table 4.16 continued overleaf

Table 4.16 continued Overview of studies on toxicokinetics and distribution: dermal route

Substance	Species	Method	Endpoint	Reference	Reliability1
^{14}C-4,4'-MDI	Skin: guinea pig, rat, human	*In vitro* absorption	No ^{14}C absorption through human skin during 54 hours exposure. Rat and guinea pig skin: minimal and slow absorption.	Clowes, 1997	3

1 Reliability key: 1 = method and description are in accordance with test guidelines
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Inhalation

Table 4.17 Overview of studies on toxicokinetics and distribution: inhalation route

Substance	Species	Method	Endpoint	Reference	Reliability[1]
^{14}C-4,4'-MDI in benzene	Rat	Toxicokinetics, distribution 15-minute. inhalation of aerosol	Faecal > urinary elimination. 70% of absorbed dose eliminated after 4 days. ^{14}C-MDI uniformly distributed predominance for lungs, muscle, kidneys, digestive tract.	Lab d' Etudes, 1977	3
^{14}C-4,4'-MDI	Rat	Biochemical and histoautoradiographic characterisation of the distribution of ^{14}C 4-hour inhalation	^{14}C level directly related to exposure conc. Minimum plateau of ^{14}C at 120 hours post-exposure. Predominant reaction: conjugation of MDI with proteins. No changes in airway morphology.	Kennedy and Brown, 1998	3
4,4'-MDI	Rat	Determination of haemoglobin adducts and urinary metabolites after a chronic inhalation study (2 years)	Arylamine-specific adducts in urine	Sepai et al., 1995b	2
4,4'-MDI	Rat	Identification of isocyanate-specific adducts in blood after a chronic inhalation study (2 years)	Isocyanate-specific adducts at least 12 times more abundant than the arylamine-specific adduct in blood.	Sabbioni et al., 2000	3
TDI	Rat	Investigation of haemoglobin or protein adduct formation	GSH transfer mechanism	Day et al., 1997	3

Table 4.17 continued overleaf

Table 4.17 continued Overview of studies on toxicokinetics and distribution: inhalation route

Substance	Species	Method	Endpoint	Reference	Reliability1
^{14}C-4,4'-MDI	Rat	Full inhalation metabolism/toxicokinetics/distribution study, part 1: excretion and distribution	Most of the systemically available dose was excreted via bile, small part excreted in urine. ^{14}C widely distributed, respiratory and excretory organs containing highest conc. of ^{14}C. Low levels of residues in all tissues after 168 hours.	Gledhill, 2001a; Gledhill et al., 2005	1
^{14}C-4,4'-MDI	Rat	Full inhalation metabolism/toxicokinetics/distribution study, part 2: metabolism.	A proportion of the MDI dose (10%) is converted to metabolites via intermediary formation of an amine group which is rapidly acetylated. No diamine detected.	Gledhill, 2001b; Gledhill et al., 2005	1
4,4'-MDI	Rat	Chronic inhalation study	Transplacental transition of MDI or degradation products	Bartsch et al., 1996	3
Polymeric MDI	Rat	Subacute inhalation study, biomonitoring	Sensitivity of markers of exposure and effects: biomonitoring is powerful for comparative dosimetry of well-defined exposure regimens.	Pauluhn, 2002b	3
Polymeric MDI	Rat	Acute inhalation/dermal study, biomonitoring	Kind and yield of biomarkers are dependent on the route of exposure and differ markedly for MDI and MDA:	Pauluhn and Lewalter, 2002	3
MDI no more data	Human	Biomonitoring: Hb adducts and urine metabolites	Detection of MDI exposure: urinary metabolites > Hb adducts	Schütze et al., 1995	2
MDI no more data	Human	Case report: biomonitoring	Urine: $T_{1/2}$ MDA = 70-80 hours. Blood: $T_{1/2}$ MDA = 21 days	Skarping et al., 1995	3
4,4'-MDI	Human	Biomonitoring	Urinary base-extractable metabolites: 0.035 to 0.83 pmol MDA/ml; AcMDA: 0.13 to 7.61 pmol/ml; MDA as Hb adduct: 70-710 fmol/g Hb; plasma MDA levels: 0.25-5.4 pmol/ml. 120 fmol/ml covalently bound to albumin.	Sepai et al., 1995a	2
MDI no more data	Human	Biomonitoring	MDA in plasma: 18/30 workers Plasma conc.: 0.05-8.48 µg/l MDA in urine: 4/30 workers	Dalene et al., 1996	3

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Other routes

Table 4.18 Overview of studies on toxicokinetics and distribution: other routes

Substance	Species	Method	Endpoint	Reference	Reliability[1]
^{14}C-4,4'-MDI	Rat	Toxicokinetic study, single intramuscular injection: excretion	Blood: $T_{1/2}$ diffus.: 12 hours, $T_{1/2}$ elimin.: 78 hours, Faecal > urinary elimination.	Lab. D'Etudes, 1976	3

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4.1.2.2 Acute toxicity

4.1.2.2.1 Studies in animals

Oral

No death occurred in groups of 5 male albino rats treated by gavage with single doses of 1,000, 2,150, 5,000 and 10,000 mg/kg polymeric MDI (Wazeter, 1964a). The MDI was administered in the pure form as received. The animals were observed for mortality and pharmacodynamic and/or toxic effects at the following intervals: immediately, at 1, 4 and 24 hours and once daily thereafter for 13 days. At the termination of the 14-day period, all surviving animals were weighed, sacrificed by decapitation and necropsied. All weight comparisons were made to a control colony of non-treated animals of the same weight range and strain in these laboratories. Weight loss was considered to be significant when it exceeded two standard deviations of the mean of a control colony of the same strain of rats of comparable age.

All rats at all dose levels gained weight normally throughout. At 5,000 mg/kg one rat displayed salivation, nasal discharge, and an abnormal yellow urine excretion during the first 24 hours. At 10,000 mg/kg two rats showed slight salivation 1 hour post compound administration. Necropsy did not reveal any abnormalities. The median oral lethal dose (LD_{50}) obtained is > 10,000mg/kg.

Acute toxicological investigations were conducted after oral administration of 2,000 mg/kg bw generic MDI, dissolved in peanut oil, to male and female Wistar rats (Bomhard, 1990). During the 14-day observation period no death occurred nor was the body-weight development affected. The LD_{50} value was not exactly determined but was greater than 2,000 mg/kg bw (limit test). All animals had increased salivation half an hour after administration, which disappeared an hour after administration. The animals' sacrifices at the end of the study period did not show noticeable gross pathological findings.

An LD_{50} of 31,600 mg/kg is entered in the Kirk-Othmer Encyclopaedia of Chemical Technology (Chadwick and Cleveland, 1981) This LD_{50} was set for MDI at 25% in corn oil (no more data).

This can be compared with the single oral lethal dose of 31,600 mg/kg from the Mobay Chemical Co. study (1961). In this briefly reported investigation, MDI at 25% corn oil was fed to rats in varying doses up to 31.6 g MDI/kg. The highest dose produced one death from a single dose, possibly due to overloading of the stomach.

In a very brief abstract (ACGIH, 1980) it is said that a single oral dose of 4.7 g/kg MDI (no more data) failed to kill rats. No further details were available.

Dermal

In the acute dermal toxicity study done by Wazeter et al. (1964d), albino rabbits were used in 4 groups of 4 evenly distributed as to sex. The dorsal skin was clipped closely, and for half of the rabbits in each group an area of approximately 3 in square (7.06 cm square) was abraded by producing shallow incisions with a scalpel blade. Food and water were available ad libitum. Polymeric MDI was applied in the liquid form, as received, at the dose levels of 2.5, 3.9, 6.0 and 9.4 g/kg of body weight. The animals were confined in immobilising holders for 24 hours with their backs covered in rubberised cloth. The back of each rabbit was then washed in lukewarm water. The animals were examined daily for a total of 14 days.

All animals showed either a body weight gain, or maintained body weight during the study, with the exception of 3 showing a slight weight loss unrelated to application of the test substance. The dermal irritation noted was slight in all instances. The slight erythematic produced initially at all dosage levels was no longer present after 7 days. All animals at all dosage levels exhibited a slight coriaceousness which in most instances continued throughout the 14 day observation period. Transient atonia was observed in a few animals at the three high dosage levels; however, this again was slight in nature. One animal at the high dosage level exhibited slight oedema during the first and second day of the study. There was no desquamation or fissuring noted with this compound. All the rabbits appeared essentially normal throughout the study with the exception of the mentioned irritation. There were no deaths and necropsy did not reveal any abnormalities. Sub-endocardial haemorrhages, apparently dose related, were of questionable significance due to the sacrifice by air embolisation. No LD_{50} could be obtained from this study, and it was therefore > 9.4 g/kg body weight.

In a review (Woolrich, 1982) a LD_{50}(rabbit) of > 6.2g/kg is reported for polymeric MDI, without supporting data.

The only other information available is a very brief report (Mobay Chemical Co., 1961) where a dermal LD_{50} of 10g/kg is stated for MDI (without data) at 25% in corn oil.

Inhalation

The acute inhalation toxicity of polymeric MDI was studied by Appelman and de Jong (1982a) by exposing young, adult SPF-bred Wistar rats in five groups, each containing 10 males and 10 females. Each group was sub-divided into sub-groups containing 5 rats of each sex. Each group was exposed, whole body, to a different concentration of the aerosol of the test substance. No control group was included. After exposure (4 hours) one sub-group from each group was killed, and another was observed for two weeks. Deaths, body weights and gross pathology were recorded. The method used was essentially that of Directive 84/449/EEC.B.2. Oil Red (0.5% w/w) was added to MDI to facilitate the spectrophotometric determination of the concentration of MDI in the exposure atmosphere. 95% of the particles were < 5 μm.

The analysed mean concentrations of MDI were 384, 418, 500 and 523 mg/m^3. The concentration achieved for the fifth group was outside the required range. Since the time of this study it has been recognised that the validity of the analytical method for MDI was questionable.

All mortalities occurred within 2 days after the end of the exposure. Both males and females lost body weight during the first 2-4 days of the observation period and then gained weight again.

The animals killed at the end of exposure to 523, 418 and 384 mg/m^3 revealed some haemorrhages or oedema of the lungs. Most animals exposed to 523 mg/m^3 had greyish and unusually wet lungs. A few animals, which died during, or were killed at the end of the observation period, had haemorrhages of the lungs. This was particularly after exposure to 418 mg/m^3. The LC_{50} (4 hours) stated in this study (Appelman and de Jong, 1982a) is 490 mg/m^3 with confidence limits of 440 and 540 mg/m^3.

Another study (Appelman and de Jong, 1982b) showed that only very small amounts of polymeric MDI components could be recovered from the fur of rats. Ingestion of MDI was not expected to contribute significantly to the effects in the above mentioned study.

In a study with a homologous mixture of MDI, Desmodur VL at 40% in 1:1 xylene: ethylene glycol acetate (Bunge et al., 1977) male and female albino rats were exposed to a MDI aerosol. Oil red was added at 0.05% solids to allow analysis of the exposure atmosphere. The parameters of toxic action were the number of animals that died, and the number showing toxic symptoms. The exposure was followed by a 4-week observation period after which the surviving animals were killed. The rats that died, and those which were killed, were autopsied. The LC_{50} (4 hours) was calculated to be 369 mg/m^3 for males and 380 mg/m^3 for females.

An early series of studies were mostly preliminary in nature. Albino rats were exposed collectively at the various dose levels for periods of 1 hour (Wazeter, 1965), 6 hours (Wazeter, 1964b), or 8 hours (Wazeter, 1964c). For these studies pure distilled monomeric 4,4'-MDI or polymeric MDI was used.

The test atmospheres were generated either by passing air through the heated test substance, or by addition of the test substance to a pre-heated flask. In the final study temperatures as high as 150 and 200°C were used. Since the vapour pressure of monomeric 4,4'-MDI is of the order of 10^{-5} mm Hg at 25°C, the test substance was presumably present as an aerosol. In the reports there is mention of fall-out and fogging in the exposure chamber.

With the exception of the final study, these studies did not generate LC_{50} values. The final study itself did not give an exact LC_{50}, as it could not be calculated from the data (Wazeter, 1965). It was said that inspection of the data gave an approximate LC_{50}(1 hour) of 172-187 mg/m^3 for monomeric 4,4'-MDI, but examination of the data reveals a clear dichotomy. For polymeric MDI no LC_{50} could be achieved. At MDI concentrations of 0.6, 81, 162 and 172 mg/m^3 there were no deaths in any of the groups of 6 rats, and no gross lesions were observed at necropsy. At 187 mg/m^3 and higher concentrations, there were deaths. The 4 deaths at 187 mg/m^3, 3 overnight and 1 at 26 hours post-exposure, were in marked contrast to the lack of deaths at 172 mg/m^3.

It has to be noted that the artificially generated aerosols required to conduct animal studies are considerably in excess of what is likely to occur in human exposures. Indeed, as reported in the ISOPA review (1998) the saturated vapour concentration is not exceeded and hence aerosols cannot be formed under normal use. While this has to be taken into account, it must be mentioned too that the inherent purpose of toxicity testing using animals is not to reproduce human exposure exactly, but to evaluate the potential of chemicals to cause damage.

The acute inhalation LC_{50} values reported in the literature vary widely. The very low vapour pressure of MDI at ambient temperature makes it very difficult to generate an atmosphere having sufficient concentration to cause any toxic effects. The International Isocyanate Institute Inc. developed a technique to create reproducible atmospheres of respirable aerosols of MDI for inhalation tests. The derived aerosol comprised a > 95% respirable fraction. The particle size distribution of 2.1 μm MMAD, having a GSD of 1.6, meets the current international recognised criteria for acute inhalation studies on rats. However, both the aerosol developed and the

conditions required to achieve the LC_{50} (4 hours, rat) of 490 mg/m^3 are artificial and not normally experienced in actual handling and use. According to information obtained from industry, typically nozzles in use for MDI-based spray applications (high pressure or airless) have MMADs of 40-120 µm and, moreover, MDI is never present alone but in combination with other compounds, which reduces the concentration of MDI with time. Consequently, in a recent ISOPA review (1998), about the evaluation of acute inhalation toxicity, it is claimed that "There is virtually no overlapping [between experimental aerosols and industrial practice] in the respirable region of the aerosols." In other words, due to the physical properties of these aerosols and the high settling velocity of particles generated under real life conditions, it may be considered that there is presently no potential of exposure to acutely toxic concentrations or doses to such aerosols. Nevertheless, possible changes in technology, either intentional or through misuse, cannot be excluded in the future.

4.1.2.2.2 Studies in human

A case history has been recently published of a worker who apparently became sensitised to MDI after a single acute high (but unknown) level inhalation of MDI, and who subsequently could no longer tolerate "subirritant" concentrations of MDI (Leroyer et al., 1998). An accidental spill of a large volume of solvent containing MDI occurred in the work area of a 54-year-old man. He was off duty during the spill and returned to work 48 hours later. There was a strong irritant smell in the plant and within one hour he experienced a headache, sore throat, cough, and chest tightness. Similar symptoms were reported by other workers. His chest symptoms increased gradually during the next month. He then noticed that wheezing and chest tightness worsened at work and improved during the weekends. An occupational challenge test with MDI (0.15 mg/m³; 4, 30, 60 minutes) demonstrated a late asthmatic response to MDI on the 60 minute challenge day.

4.1.2.2.3 Summary

There is very little information available on the effects of acute exposure to MDI in humans. However, the information available from animal studies clearly demonstrates that MDI has a very high oral rat LD_{50}: more than 10 g/kg for polymeric MDI and 31.6 g/kg for monomeric 4,4'-MDI. A dermal rabbit LD_{50} of 10 g/kg or more has been reported. Assessment of the available acute toxicity data indicates that inhalation exposure to respirable aerosols of MDI results in toxicity confined predominantly to the respiratory tract. The LC_{50} (rat, 4 hours) values reported are in the range of 369-490 mg/m^3. Strictly speaking MDI should be classified as toxic by inhalation on the basis of a 4-hour LC_{50} of 490 mg/m^3. However, a consensus was reached among European experts (Directive 67/548/EEC; 25th ATP, i.e. Dir. 98/8/EC, O.J.30.12.1998) to consider this value as irrelevant in terms of real-life exposure, because such high values are said not to be achievable except under experimental testing conditions. This pragmatic reasoning is acceptable provided that such high concentrations are indeed never achieved, even through misuse or (further) technological changes in work processes. Consequently it is proposed to classify MDI as harmful by inhalation.

Taken together, in terms of pure hazard characterisation MDI is toxic by inhalation. However, if one considers the exposure assessment, it is reasonable to consider MDI as harmful only and to apply the risk management phrase 'harmful by inhalation'.

Oral

Table 4.19 Overview of studies on acute toxicity: oral route

Substance	Species	Endpoint (LD_{50})	Reference	Reliability [1]
Monomeric 4,4'-MDI	Rat	31.6 g/kg (corn oil)	Mobay Chemical Co., 1961	4
Polymeric MDI	Rat	> 10 g/kg	Wazeter, 1964a	3
MDI no more data	Rat	31.6 g/kg (corn oil)	Chadwick and Cleveland, 1981	4
MDI no more data	Rat	> 4.7 g/kg	ACGIH, 1980	4
Generic MDI	Rat	> 2 g/kg (peanut oil)	Bomhard, 1990	1

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Poor reliability usually relates to old studies not performed by GLP or to a lack of description of the type of MDI used.

Dermal

Table 4.20 Overview of studies on acute toxicity: dermal route

Substance	Species	Endpoint (LD_{50})	Reference	Reliability[1]
Polymeric MDI	Rabbit	> 9.4 g/kg	Wazeter, 1964d	3
Polymeric MDI	Rabbit	> 6.2 g/kg	Woolrich, 1982	4
MDI no more data	Rabbit	10 g/kg (corn oil)	Mobay Chemical Co.,1961	4

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Inhalation

Table 4.21 Overview of studies on acute toxicity: inhalation route

Substance	Species	Endpoint (LC_{50})	Reference	Reliability[1]
Monomeric 4,4'-MDI	Rat	no data	Wazeter, 1964c	3
Monomeric 4,4'-MDI	Rat	172-187 mg/m^3 (1hour)	Wazeter, 1965	3
Polymeric MDI	Rat	490 mg/m^3 (4 hours)	Appelman and de Jong, 1982a, 1982b	2

Table 4.21 continued overleaf

Table 4.21 continued Overview of studies on acute toxicity: inhalation route

Substance	Species	Endpoint (LC_{50})	Reference	Reliability1
Polymeric MDI	Rat	no data	Wazeter, 1964c	3
Polymeric MDI	Rat	no data	Wazeter, 1964b	3
Polymeric MDI	Rat	no data	Wazeter, 1965	3
MDI homologous mixture	Rat	369 mg/m^3 (4 hours)	Bunge et al., 1977	4
MDI no more data	Human	Acute headache, sore throat, cough, and chest tightness. Challenge with MDI: late asthmatic response	Leroyer et al., 1998	3

1 Reliability key: 1 = method and description are in accordance with test guidelines
2 = falling short of highest standards concerning aspects of protocol or reporting
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4.1.2.3 Irritation

4.1.2.3.1 Studies in animals

Skin

In a well conducted study by Märtins (1991), 0.5 ml generic MDI was applied under semi-occlusive dressings for 4 hours to the intact but shaved skin of a group of 6 female albino rabbits, strain HC:NZW. The animals were retained for a 14-day observation period. Skin responses were recorded using the Draize scoring system. The individual findings at the various observation times are summarised in **Table 4.22**. In this study MDI was considered as severely irritating to the skin.

Table 4.22 Test for irritant effect on the skin (exposure 4 hours)

Animal no.	Body weight (kg)	Draize grade after												Irritation index	
		1 hour		24 hours		48 hours		72 hours		7 days		14 days			
		e	o	e	o	e	o	e	o	e	o	e	o	e	o
F28	3.1	1	0	2	0	2	0	2	0	2	3	0	0	2.0	0.0
D31	3.7	1	0	2	0	1	0	1	0	2	2	2	0	1.3	0.0
D34	3.5	1	0	2	0	1	0	1	0	3	3	2	0	1.3	0.0
G25	3.4	1	0	2	2	2	3	4	3	4	4	2	2	2.7	2.7
G3	3.7	0	0	2	2	2	3	3	4	4	4	3	2	2.3	3.0
G24	3.7	0	0	2	2	3	3	3	4	4	4	3	2	2.7	3.0

e Erythema and eschar formation
o Oedema formation

The mean irritation index for erythema/eschar formation was 2.1 and the index for oedema formation was 1.5.

In another well conducted study (but no GLP), 0.5 ml monomeric 4,4'-MDI was applied under occlusive dressings for 4 hours to the intact but shaved skin of a group of 6 male albino New Zealand rabbits (Fraunhofer-Institut für Toxicologie und Aerosolforschung FhG, 1981a). In this study MDI was considered as slightly irritating to the skin with an irritation index of 0.9 (0,6-3.0 = slightly irritating).

In the acute dermal toxicity study done by Wazeter et al. (1964d), 4 groups of 4 albino rabbits were used. The dorsal skin was clipped closely, and for half the rabbits in each group an area of approximately 3 in square (7.06 cm square) was abraded by producing shallow incisions with a scalpel blade. Polymeric MDI was applied in the liquid form, as received, at the dose levels of 2.5, 3.9, 6.0 and 9.4 g/kg of body weight. The animals were confined in immobilising holders for 24 hours with their backs covered in rubberised cloth. The back of each rabbit was then washed in lukewarm water. The animals were examined daily for a total of 14 days.

The dermal irritation noted was slight in all instances. The slight erythema produced initially at all dosage levels was negative after 7 days. All animals at all dosage levels exhibited a slight coriaceousness which in most instances continued throughout the 14 day observation period. Transient atonia was observed in a few animals at the three high dosage levels; however, this again was slight in nature. One animal at the high dosage level exhibited slight oedema during the first and second day of the study. There was no desquamation or fissuring noted with this compound.

In a review made by Woolrich (1982), mild irritation, which cleared in 5 days, is noted for MDI. MDI (no details available) was applied undiluted. There was no gross pathology evident 8 days after testing. Irritation was considered minimal.

PAPI 27 polymeric MDI, a commercial mixture of MDI, was evaluated in a 4-day dermal toxicity probe study (Lockwood, 1991, unpublished report). Two male New Zealand rabbits and two male Sprague-Dawley rats each received dermal applications of 1.7 ml/kg body weight of the undiluted MDI for 6 hours a day for 4 days. One of the evaluated parameters was irritation at the dermal application site.

Irritation at the test site consisted of staining and severe induration (hardening) of the skin.

One publication states that Technical MDI is slightly irritating (Duprat et al., 1976). The Technical MDI used in this study consisted of 2 lots containing 92-94% monomeric 4,4'-MDI or about 95% monomeric 4,4'-MDI, respectively. Two groups of six female rabbits were used, one for each lot, in the Draize-Test. A small pad saturated with 0.5 ml MDI was applied to the intact or scarified skin and held in place by impermeable adhesive tape for 24 hours. The application area is not specified. Histopathological observations were made at 24, 48 and 72 hours.

MDI was found to be slightly irritating. MDI was also said to be a primary irritant causing a typical burn lesion. The irritation indices for the 2 lots of MDI were 1.6 and 2.7 (irritation index between 0 and 2 = slightly irritating; between 2 and 5 = moderately irritating). There was hyperacanthosis of the epidermis which became papillary with parakeratotic focal points, supra-epidermic inflammatory crusts, ulceration also affecting the superficial part of the dermis, and prenecrotic cells disseminated or in focal points. There was intense oedema at the dermo-epidermic junction sometimes causing blisters. Additionally there was a dense inflammatory infiltrate, sometimes polymorphous, sometimes almost composed of mononucleated cells localised in the upper half of the dermis and forming perivascular sleeves. Deep dermic cells were surrounded with a sheath of mononucleated cells. Some of the rabbits showed activation or exacerbation of the lesions after 6-7 days.

Eye

Eye irritation was observed in the earlier mentioned study made by Duprat et al. (1976). A group of six female New Zealand rabbits were used in this study. 0.1 ml of Technical MDI containing 92-95% or about 95% MDI was instilled in the lower conjunctival cul-de-sac of the left eye. The right eye acted as control.

Macroscopic observation of the cornea showed that there was no immediate modification. After 24 hours the iris was normal, there was moderate conjunctivitis most often as conjunctival hyperaemia, and one rabbit had a slight corneal epithelial abrasion. In the course of the six following days the conjunctival lesions of four rabbits were effaced. The conjunctival lesions of the two rabbits were accentuated and a rough corneal zone appeared which was still present on the seventh day.

Microscopic observation on the seventh day showed slight mixed keratitis. The corneal epithelium showed atrophied zones, exulcerated zones and acanthosic reaction focal points surrounding an infero-central ulcerated zone. The stroma contained polynuclear amphophiles and there was slight oedema. The endothelium was reactional in the lower half of the eye.

According to the used method of Kay and Calandra, the derived irritation index of 6 means that MDI is slightly irritating to the eye.

In another study (Wazeter, 1964d), three groups of three albino rabbits each were used. Polymeric MDI was instilled, 0.1 ml, into the conjunctival sac of the right eye. The left eye served as an untreated control. The treated eyes of the first were not washed, the treated eyes of the second group were washed after 2 seconds, and the treated eyes of the third group were washed after 4 seconds after instillation. Eye irritation was graded and recorded according to the method of Draize. The group average scores are summarised in **Table 4.23**.

The corneal opacity noted with exposure was of a diffuse nature, the iris remaining clearly visible. All adverse corneal effects were negative at 24 hours following application. Circumcorneal injection of the iris was observed soon after application but was absent after the 24 hours observation. The severity of the irritation to the conjunctivae was of a moderate nature and in all instances examinations made on the seventh day post-treatment were negative. Purulent ocular discharge was noted in four of the nine animals. This discharge disappeared in all animals at seven days post-application. In all instances examination with sodium fluorescein and ultraviolet light of the control and treated eyes of each animal was negative, both pre-treatment and seven days post-treatment.

Table 4.23 Eye irritation test in the rabbit (Wazeter, 1964d), group average scores

Group	Observation periods											
	Minutes		Hours			Days						
	30	90	4	8	24	2	3	4	5	6	7	8
unwashed	10.3	20.9	17.3	21.2	8.6	5.6	2.0	0	0	0	0	0
2 second wash	15.3	12.9	12.9	9.2	7.4	3.4	0.6	0.6	1.2	1.2	0	0
4 second wash	14.6	16.0	8.6	10.0	3.2	7.4	1.4	0	0	0	0	0

Monomeric 4,4'-MDI, 0.1 ml, was instilled into the conjunctival sac of each eye of 6 male albino New Zealand rabbits (FhG, 1981b). The right eye was rinsed with saline 30 seconds after instillation and the left eye remained un-rinsed. Eye irritation was assessed using the Draize

scale. An irritation index of 3.9 was found (0-10 = not irritating). In this study MDI was considered to be not irritating to the eye.

In the earlier mentioned well-conducted study by Märtins (1991), 0.1 ml of generic MDI was instilled into the conjunctival sac of one eye of each of three female albino rabbits, strain HC:NZW. The treated eye was rinsed with saline 24 hours after instillation. Eye irritation was assessed using the Draize scale. MDI was considered not to be irritating to the eye. The individual findings at the various observation times are summarised in **Table 4.24**.

Table 4.24 Test for irritant effect on the eye (exposure: 24 hours)

Animal no. (bodyweight)	Tissue	Signs	Draize grades							Irritation index
D37 (3.8 kg)	cornea	o	0	0	0	0	0	-	-	0.0
		a	0	0	0	0	0	-	-	
	fluorescein	i	-	0	-	-	-	-	-	
		a	-	0	-	-	-	-	-	
	iris		0	0	0	0	0	-	-	0.0
	conjunctivae	r	0	0	0	0	0	-	-	0.0
		s	0	0	0	0	0	-	-	0.0
	aqueous humour discharge		0	0	0	0	0	-	-	
			2	0	0	0	0	-	-	
D25 (3.6 kg)	cornea	o	0	0	0	0	0	-	-	0.0
		a	0	0	0	0	0	-	-	
	fluorescein	i	-	0	-	-	-	-	-	
		a	-	0	-	-	-	-	-	
	iris		0	0	0	0	0	-	-	0.0
	conjunctivae	r	0	0	0	0	0	-	-	0.0
		s	0	0	0	0	0	-	-	0.0
	aqueous humour discharge		0	0	0	0	0	-	-	
			2	0	0	0	0	-	-	
D26 (2.9 kg)	cornea	o	0	0	0	0	0	-	-	0.0
		a	0	0	0	0	0	-	-	
	fluorescein	i	-	0	-	-	-	-	-	
		a	-	0	-	-	-	-	-	
	iris		0	0	0	0	0	-	-	0.0
	conjunctivae	r	0	0	0	0	0	-	-	0.0
		s	0	0	0	0	0	-	-	0.0
	aqueous humour discharge		0	0	0	0	0	-	-	
			2	0	0	0	0	-	-	

o Opacity; s Swelling
a Area; - Not examined
i Intensity;
r Redness

Woolrich (1982) mentions a mild inflammation and lachrymation. No gross pathology was observed three hours after testing. Irritation was considered minimal. MDI was presumably applied in a vehicle, 10% MDI, 1 mg/eye.

Observations during a sub-chronic inhalation study with polymeric MDI, are reported by Reuzel et al. (1986). Ophthalmoscopy was performed using a Heine ophthalmoscope.

Ophthalmological examinations did not reveal treatment-related changes in rats exposed to 12 mg polymeric MDI/m^3 air. The abnormalities observed were said to be common findings in the strain of rats used and their incidences showed the usual variation.

Respiratory tract

The pulmonary irritation of MDI was studied by Weyel and Schaffer (1985). Groups of 4 male, Swiss-Webster mice were exposed for 240 minutes to 6 aerosol concentrations (6.7, 10.2, 19.6, 25.8, 40.3, 58.5 mg/m^3) of 4,4'-MDI (>99.5% purity grade). Meanwhile, the respiratory patterns and frequency of 4 mice were recorded. The concentrations of MDI in the exposure chamber were determined gravimetrically. The mass median aerodynamic diameter and geometric standard deviation for the MDI aerosol were 0.7 µm and 1.6 µm, respectively. All animals were killed 24 hours post exposure by cervical dislocation. The time-response and concentration-response relationships were determined. From these results it was determined that the magnitude of effect was dependent on both the duration of exposure and the exposure concentration. MDI acted primarily as a pulmonary irritant. For the lowest concentrations (6.7 and 10.2 mg/m^3), an increase in respiratory rate above the control was observed for almost 3 hours of the exposure, followed by a gradual decline in respiratory rate during the last hour. For the highest concentration (58.5 mg/m^3) there was only a slight increase in respiratory rate, of short duration, followed by a rapid decline during the last 3 hours. For the intermediate concentrations the respiratory rate was initially elevated above the control for approximately 1 hour and gradually declined for the last 3 hours of exposure. A plateau in response was reached during the last 30 minutes of exposure. Little or no recovery was observed during 20 minutes following each exposure. The concentration required to reduce the respiratory rate by 50% (RD_{50}) due to pulmonary irritation was 32 mg/m^3. Increases in lung weight were found for all MDI concentrations. The pulmonary irritation properties of MDI were confirmed by exposing mice via tracheal cannula to 23.6 mg/m^3 MDI (sensory irritation eliminated) and by the results of the lung weights 24 hours post exposure. In this setting, a decrease in respiratory frequency was observed and lung weight increases were seen for all MDI concentrations and resulted from development of pulmonary edema. Using the RD50 of 32 mg/m^3, the authors compared the potency of MDI with that of NO_2 as a pulmonary irritant. They suggested that an exposure limit of 0.3 mg/m^3 ($RD_{50/100}$) should prevent pulmonary irritation.

A short-term inhalation toxicity study of polymeric MDI in Wistar rats was designed to investigate both the relationship between acute irritation and alteration of surfactant activity (report: Pauluhn et al., 1998; published as Pauluhn et al., 1999). The first aspect was addressed by analysing changes in breathing patterns during an acute inhalation exposure. Five groups of male rats (n = 6) were exposed for 150 minutes to the analytically determined concentrations 0 (conditioned air), 2.4, 6.7, 15.8, and 38.7 mg/m^3 respirable pMDI aerosol. Breathing patterns were examined using nose-only exposure restrainers modified to function as flow plethysmographs. Respiratory rate and tidal volume were evaluated. The respirable pMDI aerosol caused an apneic pause between end of expiration and inspiration phase of the following breath. The respiratory rate was approximately 20% above the preexposure control values for the 15.8 and 38.7 mg/m^3 exposure groups, while in the 2.4 and 6.7 mg/m^3 groups these changes of respiratory rate were indistinguishable from the air control group. As marked concentration-

dependent effects on respiratory rate were not observed, the changes in tidal volume were used to assess the extent of pulmonary irritation. Based on the results of this 150 min single-exposure study, stimulation of pulmonary irritant receptors was assumed to occur at exposure levels in the range of 2.4 mg/m³ polymeric MDI.

The second aspect was addressed in a 2-week repeated nose-only inhalation study with mean analytical concentrations of 1.1, 3.3 and 13.7 mg polymeric MDI/m^3 (6 hours/day, 15 exposures). The results show that rats exposed to 3.3 and 13.7 mg/m³ experienced mild signs of respiratory tract irritation which appeared to exacerbate during the course of the study. According to the authors, light and transmission electron microscopy suggested that exposure to 3.3 and 13.7 mg/m³ resulted in an accumulation of refractile, yellowish-brownish material in alveolar macrophages with concomitant activation of type II pneumocytes. The authors suggested that polymeric MDI appears to interact directly with pulmonary surfactant lining fluids, the first line of pulmonary defence. This assumption is further corroborated by increased levels of intracellular phospholipids – evidenced by three independent methods, i.e., polychrome stain, determination of phosphatidylcholine and electron microscopy. Statistically significant changes in the phospholipid content of alveolar macrophages were found at levels equal to or exceeding 1.1 mg/m³ MDI. In the terminal bronchioles a concentration-dependent increase of bromodeoxyuridine-labelled epithelial cells was observed in all polymeric MDI exposure groups. The findings obtained suggest that the interaction of polymeric MDI with surfactant constituents eventually leads to intracellular precipitates originating from precipitated surfactant or surfactant-polymeric MDI complexes.

In line with Pauluhn et al. (1999), Kilgour et al. (2002) – also referred to in Section 4.1.2.8.3 partly as the CTL-report 1999 – reported an acute inhalation study combined with a subacute inhalation study (28-days) designed to evaluate early changes in the lungs of female Wistar rats resulting from exposure to polymeric MDI.

In the acute inhalation study, groups of 40 female rats were exposed (nose-only) to target concentrations of 0, 10, 30, or 100 mg/m³ polymeric MDI for 6 hours. At 1, 3, 10, or 30 days following exposure, 5 rats from each group were taken for analysis of lung lavage components and 5 for pathological examination. Acute exposures produced clinical signs in all animals that were consistent with exposure to irritant aerosols (abnormal respiratory noise, breathing rate reduced and depth increased, mucous secretions from the nose). An exposure concentration-related body weight loss and increase in lung weight were seen post-exposure, with complete recovery by day 10. Immediately following exposure there were increases in total cells, total protein, alkaline phosphatase, NAG (N-acetyl-β-glucosaminidase) and some indication of increased lactate dehydrogenase (LDH) activity in lung lavage fluid. By day 3 post-exposure, further increases were apparent in total cell counts. LDH activity was elevated in all groups to a greater extent than on day 1 post-exposure, although alkaline phosphatase and NAG activity had returned to control levels. Increases in cell replication became apparent in both the terminal bronchioles and centro-acinar alveolar regions examined, the response being concentration-dependent, correlating with the concentration-dependent bronchiolar hyperplasia seen histologically and type II cell hyperplasia identified by electron microscopy. By day 10 post-exposure, most of the measured parameters had returned to control levels. Cell proliferation was still slightly higher than control levels in the 30 mg/m³ group. At the light microscopy level, macrophage accumulations were still evident in animals exposed to 10 mg/m³ only, epithelialisation of the alveoli was present in animals exposed to 30 and 100 mg/m³ and thickening of the alveolar wall and ducts were evident in animals exposed to all concentrations, although generalised effects had resolved to a large extent. By day 30 post-exposure, lung weights, lung lavage parameters, cell proliferation and ultrastructural appearance had returned to

normal at all exposure concentrations. Some slight epithelialisation of the alveolar duct and cell exudate in the lumen was still evident at low incidence in the 100 mg/m³ group, but all other effects had recovered. The time course of changes in the lung over the initial days following exposure consisted of a pattern of initial toxicity, rapid and heavy influx of inflammatory cells and soluble markers of inflammation and cell damage, increased lung surfactant, a subsequent recovery and epithelial proliferative phase and, finally, a return to the normal status quo of the lung. During these stages there was evidence of perturbation of lung surfactant homeostasis, demonstrated by increased amounts of crystalline surfactant and increased number and size of lamellar bodies within type II alveolar cells.

Repeated exposure over 28 days to 1, 4, or 10 mg/m³ polymeric MDI (6 hours/day, 5 days/week, 4 weeks, nose-only, groups of 30 female Wistar rats) produced no clinical signs or body weight changes, but an increase in lung weight was seen in animals exposed to 10 mg/m³ (35%) which resolved following the 30-day recovery period. Other effects seen were again consistent with exposure to irritant aerosols, but were less severe than those seen in the acute study. Analysis of bronchoalveolar lavage fluid showed changes in the majority of parameters at 10 mg/m³. Total cell count was increased statistically significant and this was accounted for by increases in alveolar macrophages, PMNs (polymorphonuclear cells) and lymphocytes/other cell types. At both 4 and 10 mg/m³ polymeric MDI increased numbers of 'foamy' macrophages in lung lavage cell pellet correlated with the increased phospholipid content of the pellet. Changes in lung lavage biochemical parameters (10 mg/m³: moderate increases only in total protein, LDH, alkaline phosphatase and phospholipids, no effect on NAG) and electron microscopic evidence again suggested perturbations in surfactant homeostasis. In alveolar macrophages, minimal to slight increases in lamellar surfactant were with minimal and moderate increases in amorphous surfactant in animals exposed to 10 mg/m³. In the alveolar lumina, compound-related increases in the amount of crystalline and lamellar surfactant were associated with the minimal to moderate increases in cell debris noted in animals exposed to 4 or 10 mg/m³. At 1 mg/m³, there was also some evidence of effect on surfactant homeostasis, with small increases in number and size of type II cell lamellar bodies and similar increases in amorphous, crystalline and lamellar surfactant in the alveolar lumina. Histologically, bronchiolitis and thickening of the central acinar regions was seen at 4 and 10 mg/m³, reflecting changes in cell proliferation in the terminal bronchioles and centro-acinar regions. In animals exposed to 1 mg/m³ polymeric MDI, 1/5 animals showed bronchiolitis. Almost all effects seen had recovered by day 30 post-exposure. Although, after the recovery phase, alveolar macrophages containing a yellow pigment were still present in the interstitium in all animals that had been exposed to 10 mg/m³ polymeric MDI but were absent in animals exposed to 1 or 4 mg/m³ polymeric MDI. In addition, 1/5 animals exposed to 10 mg/m³ polymeric MDI still had bronchiolitis and centro-acinar thickening, but at a reduced severity and distribution. The results are consistent with pulmonary/cellular stress in response to chemically reactive particulates. These findings suggest that an exposure concentration of 1 mg/m³ (duration of exposure 6 hours/day) for 28 days, caused non-specific cell proliferation of Type II pneumocytes.

In summary, according to the authors, exposure of rats to respirable aerosols of polymeric MDI for single acute or repeated subacute exposures resulted in a pattern of lung responses that is entirely consistent with exposure to irritant aerosols.

According to the rapporteur, in the subacute study, 1 mg/m³ is the LOAEL for effects on surfactant homeostasis (NOAEL< 1 mg/m³) and (reversible) bronchiolitis, whereas the NOAEL for pneumonitis is less than 10 mg/m³.

Pauluhn (2000) examined the acute pulmonary response of female Wistar rats (n = 6, except for the 20 mg MDI/m³ concentration n = 7) exposed nose-only to respirable polymeric MDI aerosol

(nebulised). This study investigated the time course of the relationship between acute pulmonary irritation and ensuing disturbances of the air/blood barrier in rats exposed to concentrations of 0 (conditioned dry air), 0.7, 2.4, 8, or 20 mg MDI/m³. The total duration of exposure was 6 hours. The time-response relationship of MDI-induced acute lung injury was examined at 0 hours (directly after cessation of exposure), 3 hours, 1 day, 3 days, and 7 days after exposure. Bronchoalveolar lavage (BAL) fluid was analysed for markers indicative of injury of the bronchoalveolar region (angiotensin-converting enzyme, protein, alkaline phosphatase, lactate dehydrogenase, γ-glutamyltranspeptidase, and sialic acid). Phosphatidylcholine and acid phosphatase were determined in BAL fluid and cells. Glutathione was determined in BAL fluid and lung tissue. This analysis revealed no latent period of effects except a delayed influx of cells and increased lung weights on post exposure days 1 and 3. Markedly loaded BAL cells with phosphatidylcholine were observed on day 1 only. In most instances, changes returned to the level of the air-exposed controls on day 7, except increased glutathione in lung tissue. The findings suggested that the most sensitive markers of dysfunction of the air/blood barrier were angiotensin-converting enzyme, protein, and alkaline phosphatase. The statistically significant increase in intracellular phosphatidylcholine and decreased intracellular acid phosphatase on the exposure day suggested that increased amounts of phospholipids are phagocytised by alveolar macrophages, associated with protracted lysosomal catabolism. Partially glutathione-depleted rats exposed to 20 mg/m³ experienced a more pronounced increase in BAL protein than normal rats. Pauluhn (2000) suggested that respirable polymeric MDI aerosol interacts directly with the air/blood barrier causing increased extravasation of plasma constituents as a result of increased permeability of capillary endothelial cells. A transient dysfunction of the pulmonary epithelial barrier occurred at a level as low as 0.7 mg/m³ and was interpreted as a dysfunction of pulmonary surfactant. Such dysfunction is thought to correspond to a physiological response (Pauluhn, personal communication).

In a position paper written in the wake of discussions about the interpretation of the above results, industry concluded from the studies quoted above that single or repeat (sub-acute) exposure to highly respirable polymeric MDI aerosols at high concentrations results in lung effects consistent with exposure to an irritant particulate but that recovery occurs upon cessation of exposure. No progressive or adverse effects should result from such exposures. Industry concluded that following acute 1 · 6-hour exposure to MDI aerosol, the NOAEL is 0.7 mg MDI/m³ air whereas the 2.4 mg MDI/m³ air, caused borderline biochemical effects (= LOAEL).

However, in the opinion of the rapporteur, the concentration of 0.7 mg MDI/m³ air which caused an increase in protein and angiotensin-converting enzyme (ACE) in the bronchoalveolar lavage 3 hours and one day after a six-hour exposure is a LOAEL. Admittedly, the exact biological significance of such transient increase in protein and ACE in the bronchoalveolar lavage is not known, but such changes are indicative of an increase in the permeability of the alveolar-capillary barrier. The author of the study (Pauluhn, 2000) himself uses phrases such as “transient disturbance of the air/blood barrier function” and “transient dysfunction of the pulmonary epithelial barrier” occurring at “concentrations exceeding the buffering capacity of the pulmonary lining fluids” and leading to “[depletion] of [the lungs’] own level of protection”. Such effects may or may not be adaptive, but they are indicative of injury, even if such injury appears to be rapidly reversible. The question remains, therefore, whether this LOAEL is far above the NOAEL. The latter question is addressed in part in a subsequent publication by the same author (Pauluhn, 2002b). In this study, the relative acute pulmonary irritant potencies and especially the pulmonary irritant threshold concentrations of pMDI (NCO content ≈ 31%) and HDI-IC aerosols were compared in female Wistar rats. The findings reported and discussed in this article concern 1/ results previously published on pMDI (Pauluhn, 2000) and HDI-IC; 2/new

data concerning the validity of the concentration x time concept for inhaled pMDI; and 3/new data concerning repeated exposures to pMDI aerosols. Rats were exposed by directed-flow nose-only inhalation to analytically determined breathing zone concentrations of aerosolised polyisocyanate using either single or repeated-exposure regimens. Control rats were exposed to conditioned, dry air. In all studies, the number of rats exposed was a multiple of six rats per serial sacrifice used for bronchoalveolar lavage and the number of sacrifices. Bronchoalveolar lavage determinations included total cell count of cells in bronchoalveolar fluid (BALF), total protein, angiotensin-converting enzyme (ACE), and lactate dehydrogenase (LDH). The validity of the concentration · time (C · t) concept was addressed in rats exposed to concentrations from 3.4 to 58.1 pMDI/m³ and exposure durations of 6 hours to 23 minutes, respectively (C · t ≈ 1,200 mg/m³). One additional group of rats was exposed to 2.7 mg MDA/m³ for 1 · 6 hours (represents the equimolar C · t product). With regard to lung weights and total cell count in BALF, significant changes relative to the air control group were not observed. In the pMDI groups, total protein in BALF was increased by 50% above control. Except for the 23 minutes · 58.1 mg/m³ group, changes were significantly different from the control group. For all parameters examined, MDA-exposed rats were indistinguishable from the air control group, suggesting that the increase in BALF protein was related to pMDI rather than the putative degradation product MDA. To allow a better appreciation of relative potencies of pMDI and HDI-IC aerosols, data were normalised to the means of the respective controls (=100%), one control group for each compound, and serial sacrifice. For both types of polyisocyanate aerosols a clear concentration-effect relationship could be established for BALF ACE and total protein. For protein and ACE in BALF, the data obtained at 3 hours and approximately 18 hours (post exposure day 1) were combined for each polyisocyanate because of the similar magnitude of changes. However, scrutiny of the data reveals that this was not quite true in the case of protein concentration after exposure to pMDI (approximately 50% increase, $p<0.01$, above control at 3 hours, and no change at all at 1 day), thus leading to a slight overestimation of the slope for the pMDI data. This comparison revealed that the slopes of pMDI and HDI-IC were different. When extrapolated to the level of the controls, the single-exposure NOEL for pMDI corresponded to ≈ 0.5 mg/m³ (with 95% confidence limits of approximately 0.3 mg/m³ to 0.8 mg/m³). Rats repeatedly exposed to 12.9 mg pMDI/m³ (6 hours/day, 5 days/week for 14 days), displayed signs of marked respiratory distress and significantly decreased body weights and were essentially normal within a few days post exposure. Two out of 42 rats of the pMDI group succumbed, one on day 4 and the other on day 18 (mortality appeared not to be caused by the test compound). Serial sacrifices revealed significantly increased lung weights throughout the exposure period, associated with significantly increased cell numbers in BALF. Protein in BALF was significantly increased and maximal after the first exposure day and decreased toward a lower plateau during the remaining exposure period. From the fourth post exposure day onward (day 21), changes in BALF protein were indistinguishable from the control group.

In summary, results show that total protein and ACE in bronchoalveolar lavage fluid (BALF) were among the most sensitive endpoints to probe early effects caused by exposure to irritant polyisocyanate aerosols. In the repeated-exposure study, BALF protein was maximal after the first exposure day. Based on these most sensitive endpoints in BALF, an acute irritant threshold concentration of 0.5 mg/m³ was estimated for aerosols of pMDI. This subsequent estimation indicates that the LOAEL of 0.7 mg/m³ determined in the initial publication (Pauluhn, 2000) is indeed likely to be just above an estimated NOAEL of 0.5 mg/m³.

4.1.2.3.2 Studies in humans

Case reports and workplace surveys have demonstrated skin effects usually attributable to occupational exposure to MDI. It is unclear whether or not the effects seen were due to a primary irritant inflammatory response, to local cytotoxicity, and/or sensitisation.

A NIOSH-report (1994a) makes notice of skin irritation attributed to monomeric 4,4'-MDI. Mine-workers were exposed to 'Rock glue' containing monomeric 4,4'-MDI. It was mentioned that the chemical protective gloves were not routinely utilised by the employees handling and applying rock glue adhesives. Some of the exposed miners described skin contact leading to chronic skin irritation. One current worker described continuing dermatitis of several years duration with continued rock glue exposure. Several reports on unintentional eye contact with rock glue were also provided.

In another NIOSH-report (1994b), nasal and eye irritation are the two frequently reported symptoms after monomeric 4,4'-MDI exposure.

4.1.2.3.3 Summary

Based on both animal studies and human experience, MDI can be stated to be a skin and eye irritant.

Based on 'regulatory' acute toxicity studies (see Section 4.1.2.2) no conclusions can be drawn regarding the respiratory irritating properties of MDI. However, the repeated dose studies, mechanistic studies and studies in humans (see Section 4.1.2.5 and 4.1.2.6 and 4.1.2.8) do indicate that MDI causes irritation of the respiratory tract (as expected from isocyanates). A RD_{50} (mice) due to pulmonary irritation of 32 mg/m³ was found. A LOAEL (rat, 6 hours) of 0.7 mg/m³ was observed related to a transient disturbance of the air/blood barrier function, but without evidence of cytotoxicity or pulmonary function changes (Pauluhn, 2000). The findings of Pauluhn (2000) are in line with the findings of the subacute inhalation study of Kilmour et al. (2002). In the Kilmour et al. (2002) subacute study, a LOAEL of 1 mg/m³, related to the effect on the surfactant homeostasis, was observed. These transient alterations may be indicative of "pulmonary irritation", even if such injuries appear to be rapidly reversible and of no high concern. In a subsequent publication of the same author (Pauluhn, 2002b), an acute irritant threshold concentration of 0.5 mg/m³ was estimated for pMDI, based on most sensitive endpoints in BALF. The rapporteur is of the opinion that it is appropriate and prudent to use this estimated NOAEL of 0.5 mg/m³ for further risk characterisation. [12]

Based on studies in animals and studies in humans, MDI should be classified as an irritant to the skin, the eyes, and the respiratory system.

[12] As no consensus could be reached between Industry, who favoured an irritation threshold of 0.7 mg/m³, and the Rapporteur, the opinion was sought from an expert panel composed of six mutually agreed international independent experts in pulmonary toxicology or physiology, and in particular the use of BAL parameters to assess lung injury. The majority view of the panel was in support of the NOAEL of 0.5 mg/m³ as proposed by the Rapporteur. Industry agreed with the decision of the panel experts (March 2004).

Skin

Table 4.25 Summary overview of studies on skin irritation

Substance	Species	Method	Endpoint	Reference	Reliability[1]
monomeric 4,4'-MDI	Rabbit	Draize Test	Slightly irritating and lesion burns	Duprat et al., 1976	3
monomeric 4,4'-MDI	Human	Case Report	Irritating	NIOSH-Report, 1994a	4
polymeric MDI	Rabbit	Draize Test	Slightly irritating	Wazeter, 1964d	3
MDI no more data	Rabbit	Draize Test	Mild, minimal irritation	Woolrich, 1982	4
generic MDI	Rabbit	Draize Test	Severely irritating	Märtins, 1991	1
monomeric 4,4'-MDI	Rabbit	Draize Test	Slightly irritating	FhG, 1981a	3
polymeric MDI commercial mixture	Rabbit Rat	Dermal toxicity probe study	Irritation, staining and severe hardening of the skin	Lockwood, 1991 unpublished	3

1) Reliability key: 1 = method and description are in accordance with test guidelines
2 = falling short of highest standards concerning aspects of protocol or reporting
3 = method or report not in accordance with test guidelines
4 = minimal description of method and report

Eye

Table 4.26 Summary overview of studies on eye irritation

Substance	Species	Method	Endpoint	Reference	Reliability[1]
monomeric 4,4'-MDI	Rabbit	Draize Test	Slightly irritating	Duprat et al., 1976	3
monomeric 4,4'-MDI	Human	Case Report	Irritating	NIOSH-Report, 1994a	4
monomeric 4,4'-MDI	Human	Case Report	Irritating	NIOSH-Report, 1994b	3
Monomeric 4,4'-MDI	Rabbit	Draize Test	Not irritating	FhG, 1981b	3
polymeric MDI	Rabbit	Draize Test	Irritating	Wazeter, 1964e	3
polymeric MDI	Rat	Sub-chronic inhalation study	No irritation of the eyes by inhalation	Reuzel et al., 1986	2
generic MDI	Rabbit	Draize Test	Not irritating	Märtins, 1991	1
MDI no more data	Rabbit	Draize Test	Irritating	Woolrich, 1982	4

1) Reliability key: 1 = method and description are in accordance with test guidelines
2 = falling short of highest standards concerning aspects of protocol or reporting
3 = method or report not in accordance with test guidelines
4 = minimal description of method and report

Respiratory tract

Table 4.27 Summary overview of studies on respiratory tract irritation

Substance	Species	Method	Endpoint	Reference	Reliability[1]
monomeric 4,4'-MDI	Swiss-Webster mice	Respiratory pattern, RD50	RD50: 32 mg/m³ Pulmonary irritation	Weyel and Schaffer, 1985	3
polymeric MDI	Wistar rats	Respiratory rate, tidal volume	LOEL(150 minutes): 2.4 mg/m³ Stimulation of pulmonary irritation receptors	Pauluhn et al., 1998, 1999	2
polymeric MDI	Wistar rats	Broncho-alveolar lavage	LOAEL (6 hours): 0.7 mg/m³ Dysfunction of pulmonary surfactant	Pauluhn, 2000	3
Polymeric MDI	Wistar rats	Broncho-alveolar lavage	NOAEL (6hours): 0.5 mg/m³ Increased extravasation of protein into the alveoli and airways	Pauluhn, 2002b	3
Polymeric MDI	Wistar rats	Broncho-alveolar lavage	LOAEL $_{(subacute)}$: 1 mg/m³	Kilgour, 2002	3

1) Reliability key: 1 = method and description are in accordance with test guidelines
2 = falling short of highest standards concerning aspects of protocol or reporting
3 = method or report not in accordance with test guidelines
4 = minimal description of method and report

4.1.2.4 Corrosivity

The available data suggest that MDI is not corrosive.

4.1.2.5 Sensitisation

4.1.2.5.1 Studies in animals

Skin

Polymeric MDI, was assessed for sensitising properties using a modified guinea pig maximisation test of Magnusson and Kligman by Schmidt and Bomhard, 1984. MDI, 10% in paraffin oil and Freund's Complete Adjuvant were injected intracutaneously on days 0 and 7 in 20 female Pirbright White guinea pigs. Dermal provocation applications of 0.03 and 0.1% MDI in paraffin oil were made on day 21 (24 hours) and of 0.1 and 0.3% MDI in paraffin oil on day 35 (only 6 hours). A negative control group of 10 animals was used for Freund's Complete Adjuvant, a second for paraffin oil. No positive control substance was used. Polymeric MDI was not considered to be a skin sensitiser in this test.

The guinea pig maximisation test was used by Duprat et al. (1976) to assess the skin sensitisation potential of Technical MDI containing about 92-94% MDI and 'pure' MDI containing about

95% monomeric 4,4'-MDI. MDI, 5% in olive oil and 5% in Freund's Complete Adjuvant was injected intradermally on days 0 in female albino Hartley guinea pigs. On day 7, MDI 25% in Vaseline, was applied epicutaneously on the dorsum. On day 14, MDI 10% in Vaseline, was applied epicutaneously on the flanks for 24 hours.

MDI induced cutaneous sensitivity of the contact allergy type, which was maintained over several weeks. The number of animals with skin reaction at challenge showed it to be a strong allergen. Although both grades of MDI (10% in Vaseline) were classified as extreme allergens based on the number of positive reactions at challenge, these reactions were generally weak in nature.

Positive results were reported in three well conducted Mouse Ear Swelling Tests.

Monomeric 4,4'-MDI recrystallised from hexane, was used by Thorne et al. (1987). Groups of 4-5 male BALB/cBy mice were used for each dose with many replicate determinations. Acetone containing MDI was applied to the shaved and depilated abdomen of the mice. The MDI dose range was 0.6-187 mg/kg. After 4 days the mice were challenged on the right ear with acetone, and on the left with acetone containing a dose of MDI which was non-irritating and which was known not to cause ear-thickening without sensitisation. The thickness of the ears at 24 hours after challenge was compared with that immediately before challenge. A significant response was defined as an increase greater than 2 SD above the mean response of control animals which had received acetone in the sensitisation phase and were challenged with MDI. The challenge with acetone did not produce any ear swelling. The response to MDI challenge indicated a dose-response effect. At low doses ear swelling was significant. At 0.6-37 mg/kg there was an increase in thickness with increased dose. At the highest dose there was a reduced response. Cross-reactivity to TDI and other isocyanates was demonstrated.

In another Mouse Ear Swelling Test (Tanaka et al., 1987), groups of 7-9 male, 7 week old C57Bl/6 mice were used. A 1% solution of MDI (no more data) in ethyl acetate was used for both sensitisation and challenge. In this publication they studied also the transfer of MDI-induced contact sensitivity with or without T cell deletion by monoclonal anti-Thy-1,2 antibody.

The challenge solution induced ear swelling of delayed onset with its peak 24 hours after challenge. This was seen as characteristic of delayed hypersensitivity. MDI-induced sensitivity was transferable with lymph node lymphocytes from MDI-sensitised syngeneic mice. The effector cells were T cells.

In a third Mouse Ear Swelling Test done by Ishizu et al. (unpublished, 1980), pure monomeric 4,4'-MDI was administered in various solvents (dimethylsulphoxide, ethyl acetate, acetone, dichloromethane, toluene, dimethylformamide, 1:1 ethyl acetate: olive oil, 1:1 acetone:olive oil, and olive oil suspension) to male BALB/cAnNCrj mice, in groups of 10 animals. The control mice received an equivalent amount of solvent and were challenged with MDI. The thickness of the ear was determined for both ears at 48 hours postchallenge and the results were expressed as the increased percentage of the mean value of 10 mice. The significance of the difference between the mean values for the sensitised and the control mice was evaluated using the Student's t test.

Primary irritant dermatitis was produced during sensitisation by MDI in ethyl acetate, acetone, dichloromethane, toluene, 1:1 ethylacetate:olive oil, 1:1 acetone:olive oil and olive oil suspension. This did not occur with the dimethylformamide solution. It was shown elsewhere (Tsumura et al., 1980) that MDI reacts rapidly with water in dimethylformamide to give urea derivates. At 48 hours after challenge a significant and similar increase in ear thickness was

produced by 1% solutions of MDI in ethyl acetate, acetone, dichloromethane or toluene. The suspension in olive oil gave a small increase, and the 2 mixed solvents an intermediate increase. The solutions in dimethylformamide or dimethylsulphoxide did not cause sensitisation.

Respiratory sensitisation

The pulmonary irritation and hypersensitivity in Guinea Pigs exposed to MDI (no more data) aerosol was investigated by Thorne et al., 1986. In the abstract it is stated that sensitisation to MDI was induced by inhalation of 17 mg/m^3, 3 hours/day for 5 days. The evidence for sensitisation included:

1) Respiratory response to inhalation challenge with 2.5 mg/m^3 MDI. The response had a delayed onset and was characterised by an increase in respiratory frequency occurring between 5 and 9 hours post-exposure. No such response was observed when naive guinea pigs were similarly challenged.

2) Positive skin responses upon intradermal injection of an MDI-conjugate globulins.

A second abstract (Griffiths-Johnson et al., 1990), reports very briefly the sensitisation of guinea pigs resulting from inhalation of MDI (no more data) aerosol. Eight animals were sensitised by exposure to 22 mg/m^3 MDI, 3 hours/day on 5 consecutive days. Three and 5 weeks later, they were challenged with a non-irritating concentration (3-10 mg/m^3, 1 hour). None of the animals demonstrated immediate-onset responses, but 63% developed late-onset responses (LAR).Antibody determination revealed minimal titres of IgG and occasional IgE titres.

However, in several recent well conducted studies it is stated that skin contact may be an important entry of occupational respiratory hypersensitivity. In those studies the influence of the route of exposure has been investigated.

Rattray et al. (1994) exposed guinea pigs to monomeric 4,4'-MDI by intradermal injection, by topical application or by inhalation. In addition contact hypersensitivity was measured by topical challenge and antibody responses evaluated by enzyme-linked immunosorbent assay (ELISA) and passive cutaneous anaphylaxis (PCA). Attempts to sensitise guinea pigs by inhalation exposure to MDI were unsuccessful. Antibody responses and contact sensitisation were both infrequent and low grade, and no animals exhibited pulmonary responses following challenge with atmospheric MDI. It is important to emphasise that in this investigation, inhalation sensitisation was attempted with only a single concentration of MDI. It is possible that other exposure concentrations would have been effective.

In contrast, sensitisation by either i.d. injection or topical application of MDI induced antibody responses in the majority of animals. Moreover, a proportion of animals in each case exhibited pulmonary responses following subsequent challenge. These data indicate that the route of exposure influences markedly the effectiveness of sensitisation to respiratory allergens such as MDI and that skin contact may be an important cause of occupational respiratory allergy.

Pauluhn and Mohr (1994) investigated also an animal model for MDI-induced asthma. Their experimental findings suggest that elicitation of respiratory hypersensitivity is concentration-dependent and that challenge concentrations should slightly exceed the threshold concentration for irritation (~20 mg/m^3). In another publication from Pauluhn (1993), the experimental findings suggest that the pathogenesis of MDI-induced airway disease relates more to brief high-level inhalation exposures occurring during accidental spills than to repeated low-level exposures. According to the author, it would also appear that the pathogenesis of hapten induced airway hypersensitivity is not, in fact, linked to the development of hapten-specific IgG antibody. The

findings in this study suggest that hyperresponsiveness after brief high-level exposure may be closely related to epithelial damage in the airways and the inflammatory reactions that ensue. The precise mechanisms at play here are still unclear.

In another unpublished report of Pauluhn (1994), sensitisation of guinea pigs with monomeric 4,4'-MDI included single or repeated intradermal injections followed by inhalation challenge with the MDI hapten. Additionally, two acetylcholine (ACh) provocation challenges were performed; one shortly after the hapten challenge, the other one day thereafter. High titre IgG_1 anti-MDI antibody observed in MDI-induction groups proved that successful sensitisation had occurred. However, when animals were challenged with slightly irritant concentrations of MDI, the incidence of responses was indistinguishable between the groups, i.e., neither MDI-specific immediate- nor MDI-specific delayed-onset responses were observed. Also the ensuing challenge with ACh did not identify differences in airway hyperreactivity between groups. Following the second ACh challenge, performed one day after the MDI challenge, it was apparent that some animals sensitised to and challenged with MDI demonstrated an airway hyperresponsiveness. The histopathological evaluation revealed a marked influx of eosinophilic granulocytes into the airway tissues in MDI-sensitised and MDI-challenged animals.

In a second unpublished study, (Pauluhn, 1995), only a borderline sensitisation occurred after lung sensitisation with monomeric 4,4'-MDI. A single, brief high-level inhalation exposure for sensitisation and ramped concentration of the aerosolised MDI for the elicitation of respiratory sensitisation was used. The concentrations used for challenge ranged from non-irritant to slightly irritant to the respiratory tract. Mild MDI-specific immediate-onset responses were observed mainly during challenge to slightly irritant concentrations (35 mg/m^3 air). A marked increase of neutrophilic or eosinophilic granulocytes could not be established. Correspondingly, an activation of these cells could not be observed. So, animals sensitised to high concentrations of aerosolised MDI showed a mild airway hypersensitivity without concomitant influx of inflammatory cells.

The induction of IgG_1 anti-MDI antibodies in guinea pigs following brief, high-level inhalation induction exposure was also investigated (Pauluhn, 1997) for polymeric MDI. In this study also an evaluation of respiratory hyperreactivity in rats, was made. Rats and guinea pigs showed a different susceptibility to aerosolised polymeric MDI and it appears that guinea pigs are more susceptible to acute lung damage as a result of high-level short-term exposure when compared to rats. This is apparently related to a difference in deposited pulmonary dose in the two species. The tachypnoeic response observed in guinea pig could be taken as indirect evidence for lower respiratory tract damage, corroborating the notion that the airway dose in guinea pigs was higher when compared to rats. Anti-MDI IgG_1 antibody was increased in guinea pig in a concentration-dependent manner. This dose related increase was also seen in Blaikie et al., 1995. In this study they used the single intradermal injection model in the guinea pig with subsequent inhalation challenge and serological analysis. As sensitisation dose, doses from 0.0003 up to 1% MDI in olive oil were used. Above the sensitisation dose of 1% there was no further increase in IgG1 titres. A sensitising dose of 0.0003% MDI was still able to induce antibody production in some animals. Positive pulmonary reactions on inhalation challenge were observed in animals from all groups sensitised to doses of 0.1% and above. However, there was no substantial increase in the incidence or severity of these pulmonary responses with the increase in the sensitisation dose.

The impact of particle size of aerosolised (nebulised) polymeric MDI for the induction and elicitation of respiratory sensitisation was evaluated by Pauluhn et al. (2000). Four groups of 16 female guinea pigs (Clr:[HA]BR) each received either the vehicle, repeated intradermal (id) injections (3 · 0.3% MDI), one high-level inhalation exposure of 15 minutes to 135 mg MDI/m^3 air using a small aerosol (mass median aerodynamic diameter (MMAD) ≈ 1.6 μm) or large

aerosol (MMAD ≈ 3.8 µm). Three weeks later, animals were challenged subsequently with two concentrations of MDI (average concentrations 16 and 49 mg/m³ air, each for 15 minutes) and two different particle sizes, the MMAD was either ≈ 1.6 µm or ≈ 5.1 µm for the small and large-size aerosol, respectively. Respiratory sensitisation was assessed by two endpoints: the measurement of respiratory rate, and examination of influx of eosinophilic granulocytes into the mucosa and submucosa of the trachea, bronchi, and lung-associated lymph nodes. The site of recruitment of eosinophilic granulocytes into bronchial tissues was subdivided as muscularis mucosae, submucosa, and perivascular. From measurements of respiratory rate, it would appear that guinea pigs sensitised by i.d. injections or by inhalation exposure with the large aerosol tended to display a higher responsiveness than naive controls when challenged with the small aerosol. The recruitment of eosinophilic granulocytes in the bronchial tissue was greater in both inhalation induction groups as compared to the vehicle control. It appeared that there was a somewhat greater response in animals sensitised by i.d. injections or by inhalation exposure with the large aerosol and challenged with the small aerosol. Topographically, this difference was apparent only at the bronchial perivascular level and lung-associated lymph nodes, whereas at the submucosal and muscularis mucosae level the impact on particle size tended to be less pronounced. This study suggests that a brief, high level inhalation exposure of MDI aerosol caused a sensitisation of bronchial tissues in guinea pigs. According to the author, the higher sensitisation potency of the large aerosol may possibly be related to a dosimetric phenomenon because of the greater fraction of deposition of large particles within the upper respiratory tract. Challenge exposures with this type of irritant aerosol appeared to evoke more consistent effects when the MMAD was in the range of ≈ 2 rather than ≈ 5 µm.

In another set of studies, the Murine Local Lymph Node Assay (LLNA) and Mouse IgE Test were used for the identification of respiratory allergens. Dearman et al. (1992) examined the immune response in mice following topical application of MDI (no more data) in AOO (4:1 acetone:olive oil). In this study MDI caused an increase in the serum concentration of IgE and a preferential IgG_2b rather than IgG2a response. It is the authors' view that respiratory sensitisers such as MDI cause a preferential, rather than exclusive, stimulation of T_{H2} cells. Such would accommodate the fact that MDI is able to induce contact allergy. In the same study, lymphocyte proliferative responses in draining lymph nodes were measured 3 days following exposure of mice to various concentrations of MDI. MDI caused a concentration-related increase in lymph node cells. Hilton et al. (1995) found a dose-response relationship in the mouse IgE test for MDI.

Although a number of animal studies have been published for this endpoint, none are considered as validated assays to assess the potential for respiratory sensitisation or asthma in humans.

4.1.2.5.2 Studies in humans

Skin

A case report briefly describes allergic contact dermatitis from monomeric 4,4'-MDI in a moulder (Lidén, 1980). A female medical technician developed allergic contact dermatitis on her (unprotected) forearms after making moulds from MDI. Moulds were prepared under a hood where a MDI-containing product (A) and a polyalcohol (B) were mixed and poured into a special container made of plastic and rubber. During the mixing the unprotected forearms were contaminated by the moulder. After about 18 months of work, she developed contact dermatitis on her forearms. She experienced about 10 recurrences and they were always related to the mixing of the ingredients (A and B). Patch testing with the MDI-containing ingredient A (2% in methyl ethyl ketone) was strongly positive. At a serial dilution test (1, 0.5, 0.1, 0.05, 0.01, 0.005,

0.001% in methyl ethyl ketone) she was positive down to 0.01%. The manufacturer later disclosed that ingredient A contained 30-70% of MDI and that the rest were polymers of more undefined chemical structure. A considerable variation from batch to batch was also suggested.

Another case report describes allergic contact dermatitis in a cast technician (Bruynzeel et al., 1993). A nurse developed dermatitis 3 months after she started work as a full-time cast technician. The dermatitis was localised to the flexor forearms, dorsal wrists, fingers and backs of the hands. She noticed worsening after working with a fibreglass-reinforced polyurethane cast. Traditional casts of plaster of Paris did not seem to aggravate the dermatitis. During a holiday and at weekends, it greatly improved or disappeared. Wearing long rubber gloves prevented the dermatitis. Patch tests with the European standard series and series of glues, plastics and additives gave a positive reaction to diaminodiphenylmethane (MDA) 0.5% pet. Retesting with MDA gave the same result. A test with a piece of freshly prepared cast also gave a positive reaction. Tests with MDI were negative. The casting material blamed by the technician contained, in uncured form, apart from the fibreglass and small amounts of additives, isocyanate-terminated prepolymer and diphenylmethane-4'4,-diisocyanate (MDI), according to the manufacturer. It is possible that MDA was formed by hydrolysis of MDI. This illustrates the need to perform patch tests no only with isocyanates themselves but also with their corresponding aromatic amines.

Estlander et al. (1992), reported also occupational dermatitis from exposure to polyurethane chemicals. The paper summarises the results and gives detailed descriptions of 3 out of 6 patients. All 6 patients underwent extensive patch testing. The patch tests were performed with Finn Chambers on the backs of patients using 1-day or 2-day application times. The tests included the European standard series and a series of plastics and glues containing MDA 0.5% w/w pet. Freshly made test preparations of MDI and TDI of 2% pet. but also older preparations, manufactured 5.5 months and 15.5 months earlier, with 1.5% MDI pet. were used. The results suggest that when allergy to PU chemicals is suspected, patch tests should include, in addition to MDA, at least MDI and TDI 1.5-2% pet.

A case report of occupational IgE-mediated contact urticaria from monomeric 4,4'-MDI has been described by Kanerva et al. (1999). A 28-year old carpenter, who had had respiratory atopy in childhood, presented with breathing difficulties. He had worked for 1 year glueing wood onto aluminium sheets in the manufacture of panels for ships, using a 2-component polyurethane glue, containing >30% 4,4'-MDI. 2-3 months earlier, the carpenter had, for the first time, noticed work-related breathing problems simultaneously with whealing on his lower arms. On several occasions, symptoms had reappeared when he had been working on the glueing machine. Patch tests performed with a modified European standard series, a diisocyanate series, a plant series, a phenol-formaldehyde resin series, and his own MDI-containing hardener, were all negative. Pricktests with 20 common environmental allergens and natural rubber latex were carried out by standard technique, histamine hydrochloride being used as positive control. MDI, HDI and TDI conjugates with HSA were prepared. MDI-HSA induced a histamine-size reaction (diameter 4mm) and TDI-HSA a 2 mm reaction. All other pricktests were negative. Radioallergosorbent tests (RASTs), performed with MDI, TDI and HDI indicated specific IgE-mediated sensitisation. It was concluded that the carpenter had become occupationally sensitised to MDI in the glue, causing both respiratory (asthma) and skin (contact urticaria) symptoms simultaneously. The positive RASTs to TDI and HDI appeared to indicate cross-sensitivity, as no exposure to TDI and HDI had occurred.

Bernstein et al. (1993) conducted a cross-sectional study of 243 workers (100% of the workforce) exposed to MDI in a polyurethane mould plant that had been designed to minimise MDI exposure. Levels of MDI were continuously monitored and maintained below 0.05 mg/m³.

All participants were screened by questionnaire and tests for serum antibodies to MDI-HSA (MDI-human serum albumin). Of the 243 workers tested, only 2 had elevated levels of both serum specific IgE and IgG to MDI-HSA. Both had worked in the finishing area for at least 2 years, where they applied MDI resin mixtures to mend imperfections in the final product. One of the latter aforementioned workers reported immediate-onset urticaria and facial angioedema that began 3 months after he began mixing the MDI resin mixture in the finishing area. He denied MDI-associated respiratory symptoms and was the only worker to exhibit epicutaneous reactivity to MDI-HSA (5 mg/ml). Because respiratory symptoms and peak expiratory flow rate abnormalities were absent in this case, it may be that the skin was the primary route of sensitisation. The other worker was free of symptoms and had a negative result on skin test to MDI-HSA.

A new syndrome of MDI-induced cutaneous anaphylaxis was recognised in this survey, which suggested that strict control of ambient diisocyanate exposure did not prevent the rare occurrence of IgE-mediated sensitisation through the skin.

In an epidemiological study of occupational dermatitis in 5 different shoe factories, 246 workers were interviewed, examined and patch tested using standard and occupational patch test series (Mancuso et al., 1996). In two workers with allergic contact dermatitis, sensitisation to MDI was detected. 1 of 2 workers reacted simultaneously to both MDI and MDA. The other one reacted only to MDI.

Respiratory sensitisation

Isocyanates are well documented as a cause of occupational asthma (Vandenplas et al., 1993b). In addition a hypersensitivity pneumonitis type of reaction has also been reported. Vandenplas et al. (1993a), investigated nine subjects who complained of respiratory and general symptoms related to workplace exposure. All the subjects had worked in a plant where a resin based on MDI is used in the manufacture of woodchip boards. Only two of the subjects worked permanently near the production line where the MDI resin was used, whereas the others worked daily but for variable periods of time. The authors did not measure the concentrations of airborne MDI in the plant, but the results of hygiene surveys conducted on two occasions were made available by the employer. Individual and area samples at various sites around the plant were collected on glass fibre filters impregnated with methoxypyridyl piperazine and analysed by HPLC. The first survey, performed about 2 months after the introduction of the MDI resin, showed that amounts of MDI near the press slightly exceeded the recommended TLV-TWA that had been established at 0.055 mg/m³ for an 8-hour shift. Changes in work practices and engineering controls were then planned to reduce workplace exposure. Results of individual samples obtained during the second survey were below the TLV-TWA, except for the forming line operator (0.06 mg/m³).

All subjects reported respiratory symptoms of chest tightness, cough, and shortness of breath associated with an intense systemic malaise that was characterised by myalgia, chills, headaches, and nausea. Four subjects also noted wheezing. These symptoms were clearly related to workplace exposure: they appeared between 1 and 6 h after the beginning of the work shifts, persisted until late in the evening or even during the night, and did not occur on days off work. All subjects started to experience symptoms within the first 3 months of the introduction of the MDI resin to the production process. None of the subjects were taking medication on a regular basis at the time of the challenge tests. At the time of inhalation challenges, the subjects had normal baseline spirometry; total lung capacity (TLC) and vital capacity (VC) were within normal limits. Specific inhalation challenges were carried out on the subjects as outpatients 6 to 17 weeks after complete removal from the work exposure. The subjects underwent inhalation

challenges using the MDI resin for progressively increasing periods of time on separate days. Concentrations of MDI generated in the challenge room were continuously monitored during the tests using a MDA 7100 tape monitor and were kept below the recommended TLV ceiling of 0.2 mg/m³. Exposure to these subirritant amounts of MDI induced a pattern of reaction consistent with hypersensitivity pneumonitis, i.e., significant falls in both Forced Expiratory Volume in 1 second (FEV1) and Forced Vital Capacity (FVC) associated with a rise in body temperature and an increase in blood neutrophils, in all tested subjects. All subjects experienced chills, myalgia, and arthralgia 3 to 7 hours after the end of the challenge exposure to MDI. These systemic symptoms were more intense than respiratory symptoms. Bronchoalveolar lavage, performed in two subjects 24 hours after the end of challenge exposure, revealed an increase in lymphocytes and neutrophils. Specific IgG and IgE antibodies to MDI human serum albumin (HSA) conjugates were present in all subjects. The authors concluded that the MDI resin caused a hypersensitivity pneumonitis type of reaction in at least eight (4.7%) of the 167 potentially exposed workers employed in the plant. These findings indicate that in some workplaces, a hypersensitivity pneumonitis type of reaction may be a more frequent consequence of isocyanate exposure than is usually thought.

A case is described of complex reactions associated with exposure to MDI, with some immunologic observations (Littorin et al., 1994). The patient (a smoker), a mechanic whose medical history suggested repeated attacks of a work-related pulmonary or systemic disease, was examined because of acute respiratory disorder, rhinoconjunctivitis, and a late systemic reaction after polyurethane pyrolysis products, including 4,4'-MDI. According to the polyurethane conveyer-belt producer, the polyurethane was supposedly derived from TDI. To confirm the alleged exposure, the patient (now wearing a coal-filter respiratory mask) repeated, in his workshop the imprinting job he had done in the bakehouse. The smoke contained 0.17 (0.2 m above the belt) and 0.015 (respiratory zone) mg 4,4'-MDI/m³, but no TDI. Spirometry showed a partly reversible obstructive dysfunction, and a skin-prick test was positive for MDI-HSA. MDA was detected in hydrolysed serum and urine. In serum specific IgG1, IgG4 and IgE antibodies were detected. There was a very high total IgE and a moderate neutrophilia and eosinophilia. The specific antibodies declined but were still increased five years later. Furthermore, the values of circulating immune complexes were high. *In vitro*, the circulating immune complexes in serum increased after the addition of 4,4'-MDI-HSA. The patient had anti-C1q antibodies, probably accounting for part of the circulating immune complexes. In conclusion, the reactions associated with MDI exposure (in combination with exposure to pyrolysis products) had features compatible with immediate hypersensitivity and with a complement mediated immune-complex reaction.

An interesting case report describes allergic asthma due to domestic use of insulating polyurethane foam (Dietemann-Molard et al., 1991). 8 years before the study, a 38 year–old man had bronchospasm after burning polyurethane packs. After changing jobs, respiratory symptoms disappeared. His most recent complaint was an immediate asthmatic reaction while insulating a window at home with a polyurethane foam; this was followed 24 hours later by facial swelling with rash and pruritus while drilling the dry foam. Four months after this acute clinical manifestation, laboratory results showed blood eosinophilia and high levels of specific IgE against TDI and MDI. Skin-prick tests with common inhaled allergens were negative, but patch tests with the foam and MDI were strongly positive after 24 hours. Patch tests with other isocyanates were negative. A bronchial provocation test, in a 6 m³ cabin with inhalation of 5, then 15 ppb TDI during 20 minutes remained negative. A 'realistic' test with insulating foam containing 9% free MDI was strikingly positive: after 5 minutes the patient had severe bronchospasm with a 53% fall in FEV_1. After subcutaneous terbutaline, FEV_1 almost returned to the initial value. Fifteen months later, specific IgE against isocyanates had increased. The patient

admitted having slight breathlessness two or three times when painting cars with isocyanate containing paints. This case of immediate bronchial hyperreactivity to an MDI-containing foam with IgE and cell-mediated sensitisation to the same isocyanate shows the potential danger of domestic use of such polyurethane foams; this is especially true for patients with occupational or accidental sensitisation to isocyanates.

A case of occupational asthma caused by MDI cast in a 35 year-old female nurse without atopic disposition is presented by Sommer et al., 2000 (abstract). The nurse worked in an emergency room for one year (1990-1991), applying synthetic casts containing MDI 0-3 times daily. She developed rhinitis, itchy eyes and nightly wheezing during employment in the emergency room, with subsequent serious asthma attacks in 1992 and 1996. Just before the last attack, the nurse's husband had used insulation foam containing MDI. A specific bronchial provocation test was performed with MDI-based synthetic cast material. The nurse developed an asthma attack after seven hours, with a 48% drop in FEV1, indicating that MDI was the causative agent.

A study of the health of 78 workers in an iron and steel foundry in Vancouver, British Columbia, was carried out and the results compared with those found in 372 railway repair yard workers (Johnson et al., 1985). The foundry workers were exposed to PepSet, which consists of MDI and phenol formaldehyde and their decomposition products as well as to silica containing particulates. Since they worked inside one building, they were exposed to a certain extent to all the air contaminants in the foundry. Measurements of the concentrations of quartz and MDI were carried out during the health survey by the engineering section of the Workers' Compensation Board of British Columbia. For technical reasons, measurements of MDI could not be performed by personal sampling, but area sampling (with a midget impinger containing a dilute solution of hydrochloric and acetic acids) was carried out at multiple sites in the foundry. Twenty of the 48 samples collected by personal sampling had a respirable dust level above the permissible concentration. Only two of the 319 samples, however had a concentration of MDI above the permissible concentration of 0.2 mg/m³. It should be pointed out that several months before this study the foundry had installed a new ventilation system above the moulding machines. Before the installation, levels of MDI in excess of 0.2 mg/m³ were found on several occasions. A questionnaire was administered by trained interviewers, a chest radiography, allergy tests, pulmonary function tests, and methacholine inhalation tests were carried out. Compared with the controls, the foundry workers had more respiratory symptoms and a significantly lower mean FEV_1 and Forced Midexpiratory Flow Rate ($FEF_{25-75\%}$) after adjustments had been made for differences in age, height, and smoking habits. Three workers (4.8%) had radiographic evidence of pneumoconiosis and 12 (18.2%) had asthma defined as the presence of bronchial hyperreactivity, cough, and additional respiratory symptoms such as wheezing, chest tightness or breathlessness. Sensitisation to MDI is probably the cause of asthma in these workers.

A cross-sectional evaluation was performed of workers in a steel foundry in which polymeric MDI was used as a component of a binder system used to make cores and moulds (Liss et al., 1988). Preshift and postshift spirometry and clinical evaluation, including a questionnaire, were performed on 26 currently MDI exposed core- and mould-area employees (group I), on 6 workers who had previously been in the core and mould areas but were presently working in other departments in which MDI was not used (group II), and on 14 none posed plant workers to MDI (group III). Serum samples were assayed for total antibody binding, specific IgG by ELISA, and specific IgE by the RAST method to MDI-HSA. The mean duration of exposure to MDI before onset of symptoms among the currently exposed group (group I) was 8.6 years, for the formerly exposed workers (group II) it was 1.1 year. Symptoms compatible with occupational asthma were elicited from 7 (27%) of 26 group I workers and from 3 of 6 group II workers. No symptoms were reported by group III workers. Intrashift change in FEV_1 (a mean

decrease of 0.049 l) in group I workers was significantly different from that in unexposed group III workers (a mean increase of 65 ml; p=0.043). Specific IgG and total antibody responses to MDI-HSA were detected only in workers with current or former exposure to MDI. Only one worker was identified with IgE-mediated occupational asthma exhibiting a positive prick test and elevated RAST to MDI-HSA. In this occupational setting, significant clinical respiratory and immune responses in foundry workers exposed to MDI were demonstrated. These abnormalities were not detected in workers who had not been directly exposed to MDI.

Baur et al. (1996), reported 2 cases where humoral as well as cellular immune responses were seen in asthmatic isocyanate workers induced by exposure to MDI over several months or years. Two workers (23 and 28 years old smokers) developed rhinitis and bronchial asthma after occupational contact with MDI. The previously healthy 23-year old worker had to transport empty barrels containing residues of pure 4.4'-MDI and to varnish the labels in a company producing adhesives. The average direct contact with MDI vapours was 2-3 hours per day. Approximately 5 months after starting work, he developed rhinitis and bronchial asthma. The 28-year old worker worked for 9 years in a chemical company producing two-component varnishes (polyols, biuret structure consisting of HDI trimer and an MDI prepolymer) for the car industry. He was engaged in the production of polyol components and had only minimal contact with isocyanates. Four years prior to examination, he developed workplace-related rhinitis. Shortness of breath started 1 year before examination in the evenings after handling samples of the varnish produced. Positive skin prick test results for MDI-HSA and IgE antibodies to all isocyanate-HSA (MDI, TDI, HDI) conjugates were obtained in both cases, and the inhalation challenge test with MDI produced immediate and late asthmatic reactions. In the patch test and the stimulation assay of peripheral mononuclear blood cells, a specific sensitisation to MDA (in both cases) and to further amines (in one case), as well as to hydrolysates of the respective diisocyanates, was seen, which appears to be independent of the IgE response to isocyanate-HSA. The results offer evidence of IgE-mediated, as well as lymphocyte, responses induced by exposures to isocyanate products over several months or years.

Bernstein et al. (1993) conducted a cross-sectional study of 243 workers (100% of the workforce, no information on possible drop-cuts before the survey) exposed to MDI in a polyurethane mould plant that had been designed to minimise MDI exposure. Levels of MDI were continuously monitored and maintained below 0.05 mg/m³. The average duration of employment in the plant was 18.2 months. There were a wide variety of job descriptions in the moulding plant that involved varied potential for exposure to MDI (including spills). There were 147 workers on the urethane mould lines; the 96 other workers were involved with administrative, transport, or maintenance activities. In this 3-year-old plant, MDI levels were continuously monitored 24 hours per day with area samplers (MDA 7100 monitors) positioned in multiple sites where MDI was being used. During the entire 3 years that the plant had been in operation short-term exposure did not exceed the accepted threshold limit of 0.05 mg/m³. Peak levels exceeding the latter limit that could have occurred during accidental spills were not detected by the MDA 7,100 monitors at any time during the 3 years of surveillance. Some workers in the finishing area might have been exposed to heated MDI. All participants were screened by questionnaire and tests for serum antibodies to MDI-HSA. On the basis of questionnaire responses, diagnoses were derived that included occupational asthma; non-occupational asthma; work-related and non-work-related rhinitis; and lower respiratory irritant responses. Serial peak expiratory flow rate studies were performed for 2 weeks in 43 workers with and in 23 workers without lower respiratory symptoms. Results of serial peak expiratory flow rate studies were abnormal in 3 (33%) of 9 workers with occupational asthma, in 2 (50%) of 4 with non-occupational asthma, and in 2 (9%) of 23 case control subjects. A significant association was found between peak flow rate variability and a questionnaire asthma

diagnosis (χ^2 $p<0.002$). Physicians confirmed 3 cases of occupational asthma, one of which occurred in a control worker who was free of symptoms. In all three cases asthma symptoms remitted after the worker left the workplace. Serum specific IgE and IgG levels were elevated in 2 of 243 workers, one of whom was prick test positive to MDI-HSA and had had cutaneous anaphylaxis after MDI exposure. Both cases worked with the MDI-resin mixture in the finishing area where there was direct skin contact with MDI. On the basis of these cases, specific work activities associated with exposure to MDI were identified and corrective measures were instituted. Strict control and monitoring of ambient MDI exposure was associated with a low prevalence of specific sensitisation to MDI and a 'lower than expected' (in comparison with similar plants where TDI had been used) prevalence of occupational asthma. However, even then occupational asthma was not prevented entirely, since at least 3 out of 246 (or 147) subjects had developed occupational asthma over a period of 3 years or less.

A new syndrome of MDI-induced cutaneous anaphylaxis was recognised in this survey, which suggested that strict control of ambient diisocyanate exposure did not prevent the rare occurrence of IgE-mediated sensitisation through the skin.

In a study of occupational asthma among workers exposed to 4,4'-MDI, Lushniak et al. (1998) tried to verify if serum concentrations of MDI-specific IgG or IgE are sensitive biological markers of disease or of MDI exposure. The study group consisted of 9 MDI-exposed workers (8 current foamers and 1 painter who had formerly been a foamer) and 9 non-exposed workers (painters). None of the workers had previous known exposures to TDI or HDI. None of the foamers were provided with or wore respiratory protection. Air sampling for MDI and polymethylene polyphenyl isocyanate, occupational and medical histories, respiratory physical exams, pre-and postshift spirometry, and self-administered peak expiratory flow rats were performed. Serum specific IgE and IgG antibodies to an MDI-human serum albumin (HSA) conjugate were assayed by the radioallergosorbent test and the enzyme-linked immunosorbent assay, respectively, and compared to 9 non-exposed laboratory controls. Two days of air monitoring demonstrated that foamers at this plant were exposed to MDI at levels below occupational exposure limits. No definitive cases of occupational asthma were documented. Six of nine workers in the exposed group had elevated IgG antibodies specific for the MDI-HSA conjugate. Of this group, two were symptomatic and had a decreased FEV_1/FVC ratio indicative of possible asthma. The mean level of MDI-specific IgG was significantly greater among exposed workers compared to non-exposed workers and laboratory controls ($p = 0.04$). Mean levels of TDI and HDI-specific IgG were also increased. The authors concluded that serum concentrations of MDI-specific IgG appear to be a moderately sensitive biological marker of MDI exposure, but not an indicator of occupational asthma. Workers with IgG antibodies specific for one diisocyanate-HSA conjugate exhibited cross-reactivity to antigens prepared with other diisocyanates.

Several publications indicate that complex immunological reactions are involved in the sensitisation process to MDI. Humoral as well as cellular mechanisms are involved in the pathogenesis. Immediate allergic, late allergic and dual-phase responses can occur. The specific humoral response can be IgE as well as IgG mediated, but many patients with sensitisation to isocyanates have no demonstrative serum antibodies against the isocyanates. Bernstein et al. (1997) suggest an underlying genetic susceptibility.

In an attempt to try to understand the immunological basis of diisocyanate asthma, Elms et al. (2001) investigated immune cell responses to TDI, HDI and MDI, using the human monocytic cell line mono-mac-6, by measuring the production of hydrogen peroxide, and the expression of ICAM-1 (CD54) following challenge with the isocyanates and their corresponding amines. The isocyanates were dissolved in phosphate buffered saline to mimic the physiological conditions of

the lung (no more data available). The Mono-mac-6 cells were incubated for 18 hours for ICAM-1, and 5-20 minutes for the oxidative burst studies with 10 μM (maximal non-toxic concentration) of TDI, HDI, MDI, TDA, HDA, MDA, or unstimulated as a control. As a negative control, cells were also incubated with glycerol. The investigators observed an increase in the levels of intracellular peroxide, in addition to an upregulation of ICAM-1 expression ($p < 0.05$), following cell stimulation with the isocyanates, which was not apparent following stimulation with their corresponding amines. Peroxide levels were significantly increased ($p < 0.05$) in mono-mac-6 cells challenged with either TDI or MDI for 20 minutes compared to unstimulated cells, cells challenged with the corresponding amines or cells challenged with the non-sensitising glycerol. Cell challenge with amines did not induce an increase in peroxide levels that were significantly different to that of glycerol ($p > 0.05$). It was hypothesised that the production of reactive oxygen species (ROS) by monocytic cells at the site of exposure to an isocyanate may have two potential outcomes. The first is that the ROS may contribute to tissue damage at the site of inflammation. Secondly, it is possible this production of hydrogen peroxide may also induce the upregulation of adhesion markers on monocytic cells, especially ICAM-1, which may potentiate the infiltration and adhesion of cells at the site of inflammation. According to the authors, the action of isocyanates, in conjugation with data suggesting that isocyanates do not immediately hydrolyse in phosphate buffered saline (K. Jones personal communications in Elms, 2001), indicates that isocyanates may not immediately hydrolyse after inhalation and may interact and activate immune cells. However, it remains the question if for this study freshly made diisocyanate solutions were used or not: the duration of the diisocyanates in the phosphate buffered saline is not mentioned. In conclusion, the endpoints of immune cell upregulation investigated in this study were significantly more activated by isocyanate than the corresponding amine, suggesting that unreacted isocyanates could have an important role to play in the pathophysiology of isocyanate induced occupational respiratory disease.

Recently, Bernstein et al. (2002) reported that monocyte chemoattractant protein-1 (MCP-1) *in vitro* production had a sensitivity and specificity of 79% and 91% in diagnosing diisocyanate asthma. The study was performed to evaluate test characteristics of the *in vitro* MCP-1 assay compared with diisocyanate-HSA-specific IgG and IgE in identifying workers with diisocyanate asthma. MCP-1 was quantitated in peripheral blood mononuclear cell supernatants 48 hours after incubation with diisocyanate-HSA antigens. Assay results were compared with outcomes of specific inhalation challenge (SIC) testing. Of 54 diisocyanate-exposed workers evaluated, 8 (15%) had prior work exposure to TDI, 36 (67%) to HDI, and 10 (18%) to MDI. At the time of evaluation, 39 (72%) workers had current exposure or exposure within 6 months, and 15 (28%) workers had been remotely exposed (range: 7 to 93 months). Nineteen of 54 (35%) workers assayed for antibodies and MCP-1 stimulation had SIC-confirmed diisocyanate asthma. Mean MCP-1 produced by SIC-positive workers was greater than SIC-negative workers ($p \leq 0.001$). Diagnostic sensitivity, specificity, and test efficiency for specific IgG were 47%, 74%, and 65%, respectively, and for specific IgE were 21%, 89%, and 65%, respectively. It is clear that diisocyanate antigen serum-specific IgE may be meaningful, if positive, in HDI and MDI but not in TDI-exposed workers, but lacks the overall sensitivity needed for medical screening. Sensitivity, specificity, and test efficiency of the MCP-1 test were 79%, 91%, and 87%, respectively. As a reference group, a group of 9 non-asthmatic volunteers with no known previous exposure to diisocyanates underwent testing for *in vitro* MCP-1 production. These findings indicate a strong association between diisocyanate antigen enhancement of MCP-1 and diisocyanate asthma.

However, it remains to be seen whether this early promise of the *in vitro* MCP-1 test will be fulfilled in practice, and whether it can be extended with similar benefit to other causes of occupational asthma (Hendrick, 2002).

Further investigation and validation of cellular immunoassays could enable development of more sensitive and specific diagnostic tests useful in the diagnosis of occupational asthma.

4.1.2.5.3 Summary

Animal data as well as studies in humans provide clear evidence of possible skin sensitisation due to MDI. Animal studies indicate that MDI is a strong allergen. Human case reports describe the occurrence of allergic contact dermatitis due to MDI exposure.

MDI is a potential respiratory sensitiser in animals and humans. Animal studies have shown that respiratory sensitisation can be induced by skin contact with MDI. The quantitative relationships between exposures (concentration, duration, rate of exposure, route of exposure) have not been established.

At the present time it is not possible to define reliable exposure-response relationships with regard to the risk of sensitisation for MDI. The current knowledge/state of the art in this field does not yet allow to decide a threshold level for sensitisation. Because animal data support the hypothesis that respiratory hypersensitivity may be induced by skin contact and because such possibility has not been excluded in studies involving humans, it is reasonable to consider that it is not only important to reduce inhalation exposure but also to avoid skin contact.

The mechanism behind isocyanate-related hypersensitivity is still obscure. Several publications indicate that complex immunological reactions are involved in the sensitisation process to MDI. Immediate allergic, late allergic and dual-phase responses can occur. Humoral as well as cellular immunity may be involved in the pathogenesis of hypersensitivity due to isocyanates. The specific humoral response can be IgE as well as IgG mediated. Cross-reactivity with other isocyanates has been described in several publications.

<u>Skin</u>

Table 4.28 Summary overview of studies on skin sensitisation

Substance	Species	Method	Endpoint	Reference	Reliability[1]
Monomeric 4,4'-MDI	Guinea pig	Maximisation test	sensitising	Duprat et al., 1976	3
Monomeric 4,4'-MDI	Mouse	Mouse ear swelling test	sensitising	Thorne et al., 1987	3
Monomeric 4,4'-MDI	Mouse	Mouse ear swelling test	sensitising	Ishizu et al., 1980	3
Monomeric 4,4'-MDI	Human	Patch test	allergic contact dermatitis	Lidén, 1980	4
Monomeric 4,4'-MDI	Human	Patch test	contact dermatitis	Bruynzeel et al., 1993	4
Monomeric 4,4'-MDI	Human	Patch test Prick test RAST	asthma and contact urticaria	Kanerva et al., 1999	4
Monomeric 4,4'-MDI	Human	Patch test	occupational dermatitis	Estlander et al., 1992	3

Table 4.28 continued overleaf

Table 4.28 continued Summary overview of studies on skin sensitisation

Substance	Species	Method	Endpoint	Reference	Reliability1
Polymeric MDI	Guinea pig	Modified Maximisation test	not sensitising	Schmidt and Bomhard, 1984	3
MDI no more data	Mouse	Mouse ear swelling test	sensitising	Tanaka et al., 1987	3
MDI no more data	Human	Patch test	sensitising	Mancuso et al., 1996	3
MDI No more data	Human	Questionnaire Peak expiratory flow rate ELISA Prick test	MDI-induced cutaneous anaphylaxis	Bernstein et al., 1993	3

1) Reliability key: 1 = method and description are in accordance with test guidelines
2 = falling short of highest standards concerning aspects of protocol or reporting
3 = method or report not in accordance with test guidelines
4 = minimal description of method and report

Inhalation

Table 4.29 Summary overview of studies on respiratory sensitisation

Substance	Species	Method	Endpoint	Reference	Reliability[1]
Monomeric 4,4'-MDI	Guinea pig	different routes of sensitisation, inhalation challenge	sensitising	Pauluhn, 1995	2
Monomeric 4,4'-MDI	Guinea pig	different routes of sensitisation, inhalation challenge	sensitising	Rattray et al., 1994	2
Monomeric 4,4'-MDI	Guinea pig	different routes of sensitisation, inhalation challenge	sensitising	Pauluhn and Mohr, 1994	2
Monomeric 4,4'-MDI	Guinea pig	different routes of sensitisation, inhalation challenge	sensitising	Pauluhn, 1993	3
Monomeric 4,4'-MDI	Guinea pig	different routes of sensitisation, inhalation challenge	sensitising	Pauluhn, 1994	2
Monomeric 4,4'-MDI	Guinea pig	different routes of sensitisation, inhalation challenge	sensitising	Blaikie et al., 1995	2
Polymeric MDI	Guinea pig, rat	respiratory sensitisation	sensitising	Pauluhn, 1997	3

Table 4.29 continued overleaf

Table 4.29 continued Summary overview of studies on respiratory sensitisation

Substance	Species	Method	Endpoint	Reference	Reliability1
Polymeric MDI	Guinea pig	different routes of sensitisation, inhalation challenge	sensitising	Pauluhn et al., 2000	3
Polymeric MDI	Human	IgG and IgE (Rast, Elisa)	sensitising	Liss et al., 1988	3
Polymeric MDI	Human	broncial provocation with MDI; spirometry; BAL	sensitising: hyper-sensitivity pneumonitis	Vandenplas et al., 1993a	3
MDI no more data	Guinea pig	inh. sens: 22 mg/m^3, 3h/day, 5 days inh challenge: 3-10 mg/m^3, 1h	sensitising	Griffith-Johnson et al., 1990	4
MDI no more data	Guinea pig	inh. sens: 17 mg/m^3, 3h/day, 5 days inh challenge: 2.5 mg/m^3	sensitising	Thorne et al., 1986	4
MDI no more data	Mouse	local lymph node assay; mouse IgE test	sensitising	Dearman et al., 1992	2
MDI no more data	Mouse	local lymph node assay; mouse IgE test	sensitising	Hilton et al., 1985	2
MDI in PepSet	Human	radiography; spirometry	asthma	Johnson, 1985	3
MDI no more data	Human	monitoring serum/urine, spirometry, prick test	acute respiratory disorder, rhinoconjunctivitis, fever, late systemic reactions	Littorin et al., 1994	3
MDI no more data	Human	inhalation challenge, spirometry, prick test, ELISA	rhinitis, bronchial asthma, humoral and cellular immune responses	Baur et al., 1996	3
MDI no more data	Human	patchtest, bronchial provocation, spirometry	asthma	Dieteman-Molard et al., 1991	4
MDI No more data	Human	bronchial Provocation, spirometry	asthma	Sommer et al., 2000	4

Table 4.29 continued overleaf

Table 4.29 continued Summary overview of studies on respiratory sensitisation

Substance	Species	Method	Endpoint	Reference	Reliability1
MDI no more data	Human	questionnaire Peak expiratory flow rate ELISA prick test	occupational asthma	Bernstein et al., 1993	3
MDI No more data	Human	questionnaire peak expiratory flow rate RAST ELISA	occupational asthma MDI-specific IgG	Lushniak et al., 1998	3
MDI No more data	Human	spirometry, bronchial provocation, ELISA IgG and IgE, *in vitro* MCP-1 assay	diisocyanate asthma MCP-1 stimulation	Bernstein et al., 2002	3
MDI No more data	Human mono-mac-6 cell line	production of hydrogen peroxide, expression of ICAM-1	increase in intracellular peroxide, upregulation of ICAM-1 expression	Elms, et al., 2001	4

1) Reliability key: 1 = method and description are in accordance with test guidelines
2 = falling short of highest standards concerning aspects of protocol or reporting
3 = method or report not in accordance with test guidelines
4 = minimal description of method and report

4.1.2.6 Repeated dose toxicity

4.1.2.6.1 Studies in animals

Inhalation

Four groups of six rats (male albino rats, Manor Farms pathogen-free strain), were exposed for 30 minutes/day, 5 days/week for 2 weeks to polymeric MDI vapours (Wazeter, 1964e). The target concentrations were 0, 1.0, 5.2 and 10.4 mg/m^3. Complete vaporisation of the test substance was impossible.

The analysed mean concentrations of the test substance achieved were 2.0, 6.7 and 26.8 mg/m^3. The control rats and the exposed rats appeared essentially normal at all times with the exception that there was occasional slight ptyalism and increased grooming activity amongst the rats exposed to 26.8 mg/m^3 of polymeric MDI. All the rats survived. There was no significant alteration in body weight of any of the rats. Haematologic examinations (total and differential leucocyte counts and eosinophil counts) did not reveal any abnormalities that were compound related. None of the pathologic lesions observed at necropsy were compound related. Histopathologic examination of formalin-fixed haematoxylin-eosin stained, paraffin sections of the lung, liver and kidney from all rats did not reveal any compound related lesions.

In summary, in this subacute inhalation study, they made use of 'vapours', but complete vaporisation of the test substance was impossible. No adverse compound-related changes were found with respect to body weight gains, haematology or gross and microscopic pathologic examination. The NOAEL in this study was determined to be > 26.8 mg/m^3.

In another subacute inhalation toxicity study by Wazeter, 1964f, a group of 15 female, albino rats (Charles River) were exposed to polymeric MDI, 8 hours/day, 5 days/week for 4 weeks. The test atmospheres were generated by passing dried air through the hot test substance. A second group was exposed in an identical manner except that polymeric MDI was not present.

Periodic analysis gave test substance concentrations ranging from 0.06 to 2.86 mg/m^3. Successive analytical values on the same day tended to decrease. All the rats survived the 4-week study, and all appeared essentially normal throughout. No statistically or biologically significant alteration in body weight was observed during the study. Haematologic examination conducted at the end of the 4th week did not reveal any abnormalities due to the MDI. The NOAEL in this study is greater than 2.9 mg/m^3. However pathological studies were hindered by the prevalence of spontaneous respiratory disease in the rats used. The only changes possibly related to the test substance were a slight increase in the incidence and severity of tracheitis and lung petechiation in the test group.

In a more recent study, 4-week old rats (Wistar, strain: Cpb:WU) were randomly divided into 4 groups each containing 10 males and 10 females. These groups were exposed to polymeric MDI aerosol at target concentrations of 0, 2, 5, and 15 mg/m^3 air, respectively for 6 hours/day, 5 days/week over a period of 2 weeks (Reuzel, 1985a). In this study a proper distribution and stability of the polymeric MDI aerosol in the inhalation chambers could be established. The overall mean concentrations as determined by means of QCM (Quartz Crystal Microbalance) cascade were 2.18 ± 0.23, 4.88 ± 0.77, 13.55 ± 1.30 mg/m^3 air, respectively. 95% of the particles were at < 5 μm. No MDA and no phenyl isocyanate (PhI) could be detected in the test atmospheres.

Severe respiratory distress was observed in male and female rats exposed to 15 mg/m^3 polymeric MDI. Male rats exposed to 5 mg/m^3 showed similar but much less severe signs.

7 out of 10 males and 1 out of 10 females exposed to 15 mg/m^3 polymeric MDI died before the end of the study. Gross pathological examination failed to reveal changes which could be ascribed to the test material. Severe growth retardation was observed in male and female rats exposed to 15 mg/m^3 MDI and slight growth retardation in males exposed to 5 mg/m^3. Rats exposed to 15 mg/m³ showed severe depression in weight gain. In fact these animals hardly grew, whereas the controls gained nearly 100% body weight. Also in males of the mid-level group weight gain was statistically significantly lower than that of the controls. Females exposed to 2 mg/m³ showed lower weight gain than did controls. Since at the higher exposure level of 5 mg/m³ weight gain was about similar to that of the controls the differences between female controls and females of the low-level group were considered to be toxicologically insignificant. Mean absolute lung weights were decreased in the top-level group and slightly increased in males of the low-level group compared to the controls. When the lung weights were expressed relative to the body weights, it appeared that the mean lung weights in all the test groups were higher than those of the controls. There was a positive dose-response relationship both in males and females; the differences were statistically significant only in rats exposed to 15 or 5 mg polymeric MDI/m³. Based on the marginal increases in lung-to-body weight ratios it was concluded that 2 mg polymeric MDI/m^3, which was the lowest dose level examined, was an effect level.

In a subsequent subchronic inhalation study by Reuzel et al. (1985), 6-week old Wistar (Cpb:WU) rats were randomly divided into 4 groups, each containing 15 of each sex. The 4 groups were exposed to polymeric MDI aerosol at target concentrations of 0, 0.2, 1.0 and 5.0 mg/m^3 air, respectively, for 6 hours/day, 5 days/week over a period of 13 weeks. Symptomatology, body weight gain, haematology, biochemistry, urine analyses, organ weights and gross- and microscopic pathology were used criteria to disclose possible adverse effects.

The actual concentrations of polymeric MDI aerosol in the test atmospheres as determined by QCM cascade were 0.20, 1.04 and 5.03 mg/m³. Particle size determinations revealed that more than 95% of the particles had an aerodynamic diameter smaller than 5 μm.

Transient slight growth retardation was observed in male rats exposed to 5 mg/m^3 air. Haematology, blood chemistry and urinalysis did not show treatment-related effects. There were no significant differences in organ weights between the test and control groups. Gross examination at autopsy did not reveal changes which could be ascribed to the test substance. Histopathological examination revealed yellow material in the respiratory tract of rats exposed to 5 mg/m^3. Under the conditions of this test no clear adverse-effect level was determined.

The unexpected lack of adverse effects in this study gave rise to some doubt about the reliability of the data of the previous 2-week study. Furthermore, since 4-week old rats were used in the 2-week study while 6-week old rats were used in the 3-month study an age dependent susceptibility to MDI could not be excluded.

To answer this question and to confirm that mortality could occur at a level of 15 mg/m^3 a second 2-week study was conducted prior to initiation of an additional 3-month study. In this study (Reuzel, 1985b) half the number of males and females were 4 weeks and the other half 6 weeks at the start of the exposure period. The mortality data confirmed the results of the previous subacute (2-week) inhalation study. In addition, it appeared that 4-week old rats died earlier and in much greater numbers than 6-week old rats. This age-dependent difference in mortality might explain the absence of compound –related effects in the older rats of the subchronic study. Therefore the results of both studies appeared to be complementary rather than contradictory. No NOAEL was determined in this study.

In a second subchronic (3-month) study by Reuzel et al. (1986), 240 Wistar rats (strain Cpb:WU), 6-weeks old, were randomly divided into 4 groups each containing 30 males and 30 females. The aerosol target concentrations were 0, 4, 8 and 12 mg polymeric MDI/m^3. Symptomatology, ophthalmology, body weight, haematology, biochemistry, urine analyses, organ weights, gross- and microscopic pathology and lung lavages were used as criteria to disclose possible adverse effects.

The mean concentrations of polymeric MDI in the test atmospheres as determined by gravimetry were: 4.07, 8.43 and 12.25 mg/m³ air. >95% of the particles were smaller than 5 μm.

11 males and 4 females exposed to 12 mg/m^3 died during the exposure period. Deaths were not observed during the following recovery period. Severe respiratory distress was observed in rats exposed to 12 mg/m^3. Clearly less severe signs were seen in rats exposed to 8 mg/m^3. Ophthalmoscopy did not show changes which could be ascribed to exposure to MDI aerosol. In male rats growth was clearly more affected by exposure to polymeric MDI aerosol than in female rats. Depressed body weight gain was statistically significant in males exposed to 12 mg/m^3 during the exposure period when compared to the controls. In addition there was reduction in weight gain in males exposed to 8 mg/m^3. The differences with the controls, however, were statistically significant only at 7 weekly measurements which were scattered over the exposure period. During the recovery period body weight gain recovered completely in males

exposed to 8 and for a major part in males exposed to 12 mg polymeric MDI/m³. Females exposed to 12 mg polymeric MDI/m³ air exhibited a slight transient reduction in weight gain during the first 3 weeks of the exposure period. In addition body weight gain was reduced in females exposed to 8 and in males exposed to 4 mg polymeric MDI/m³ air only during the first week of the study. Haematological examinations were essentially negative. Dose-related increased creatinine values in blood plasma at the end of the post-treatment period occurred in females exposed to 8 or 12 mg/m^3. Urine analyses in treated animals were similar to those of the controls. There were no statistically significant differences in absolute organ weights between the treated groups and the control groups which could be related to treatment. However, when expressed relative to body weights the values for lung weights in both males and females exposed to 8 or 12 mg/m³ were statistically significantly greater than in controls at the end of the exposure period. The differences in relative lung weights with the controls were dose-related in males but not in females. At the end of the post-treatment period lung weights of male and female rats exposed to 8 mg and of females exposed to 12 mg polymeric MDI/m³ were comparable with those of the controls, whereas those of males exposed to 12 mg/m³ were slightly lower. Both absolute and relative weights of the other organs showed the common variation amongst the groups and were not affected by treatment. Gross examination at autopsy did not reveal changes which could be ascribed to the exposure. Treatment-related histopathological changes were found in the nasal cavity, the lungs and the mediastinal lymph nodes in all treated groups. Thinning of the layer of olfactory epithelium in the posterior part of the nasal cavity was observed in some animals in each of the treated groups but not in the controls. This change was considered a type of atrophy. Incidence and degree increased with increasing dose levels in females but not in males. The difference in incidences of the atrophy was statistically significant in both males and females exposed to 10 mg/m³ when compared with the controls. In males exposed to 12 mg/m³ and in females exposed to 8 or 12 mg/m³ the epithelial atrophy was occasionally accompanied by focal hyperplasia of basal cells. Rhinitis was found in some animals exposed to 8 or 12 mg/m³ in association with the atrophic changes. In addition, an increase in incidence and severity of nest-like infolds of the respiratory epithelium covering the nasal septum and the nasal turbinates was found in females, but not in males, exposed to 8 or 12 mg/m³ when compared with the controls. These increases were related to the dose levels. Histopathological examination revealed accumulation of macrophages containing yellow material in the lower respiratory tract and the mediastinal lymph nodes at all exposure levels. In several rats exposed to 8 or 12 mg polymeric MDI/m³ air macrophages were also found in the interstitium of the alveolar septa. Frequently this was associated with an increase incidence of a focal reaction, seen as increased septal cellularity consisting of mainly mononuclear inflammatory cells and fibroblasts. The differences in incidences of macrophage accumulations and of interstitial macrophage infiltrations between controls and the treated groups did not show a dose-relationship. Incidence and degree of the septal tissue reaction increased with increasing dose levels. The differences in incidence between controls and the test groups were already statistically significant in males exposed to 4 and in females exposed to 8 mg/m³. No evidence of tissue reaction was found in the lymph nodes. At the end of the post-treatment period changes in the nasal cavity and the lungs were still present but mostly to a lesser degree, except for the interstitial macrophage infiltration. Also in the mediastinal lymph nodes accumulations of macrophages persisted but still without tissue reactions. Except for lymphoid depletion in thymus and spleen no distinct treatment-related pathology was seen in animals that died or were killed in extremis. The cause of death could not be explained by microscopic examination. Phagocytosed material was observed at the end of the treatment and post-treatment periods in lung macrophages from rats exposed to 4 or 8 mg/m^3. Rats of the 12 mg/m^3 group were not examined due to early mortality. At the end of the post-treatment period the

phagocytotic capacity of the macrophages was lower in females exposed to 8 mg/m^3 than the controls.

It was concluded that inhalation exposure of polymeric MDI at 8 or 12 mg/m^3 for 13 weeks caused clear adverse effects and that the No-Adverse Effect Level (NOAEL) was less than, but probably close to, 4 mg/m^3.

The subacute (Reuzel, 1985a, 1985b) and subchronic (Reuzel et al., 1985, 1986) inhalation studies were looked at as a whole and published by Reuzel et al. (1994b). It was concluded by the authors that the dose-effect curve for repeated exposures of rats to respirable polymeric MDI is very steep, and that the NOAEL of polymeric MDI was 1.4 mg/m³, the actual NAEL being lower than but most probably very close to 4.1 mg/m³.

In a briefly reported subchronic study (an abstract) from Heinrich et al. (1991), female Wistar rats were exposed for 18 hours/day, 5 days/week for 90 days to an monomeric 4,4'-MDI aerosol at the concentrations 0.3, 1.0, and 3.0 mg/m^3. The observed effects were: slightly lower body weight gain; increase of the wet and dry lung weight after 1 and 3 mg/m^3 exposure; total cell count of bronchio-alveolar lavage (BAL) as well as the percentage of granulocytes and lymphocytes from the highest dose group was clearly higher, and the percentage of macrophages was reduced. Total protein, β-glucuronide and lactate dehydrogenase in BAL were also higher in the highest group. Mechanical lung function measurements using the whole body plethysmograph and the anaesthetised, spontaneously breathing rat showed a larger functional residual capacity and residual volume, decreased quasistatic lung compliance and CO diffusing capacity after 3 mg/m^3 exposure. The histopathological investigation after exposure to 1 and 3 mg/m^3 revealed submucosal infiltration of mononuclear cells, goblet-cell hyperplasia, erosion of the respiratory epithelium in nasal and paranasal sinus, hyperplasia of the bronchus associated lymphatic tissue, and inflammatory alterations in the lungs. In this study the NOAEL was set on 0.3 mg/m^3 and the LOAEL on 1.0 mg/m^3.

A chronic toxicity/carcinogenicity inhalation study was carried out by Reuzel et al. (1990, 1994a). The Wistar rats received a polymeric MDI aerosol 6 hours/day, 5 days/week during 1 (the satellite group) or 2 years. The target concentrations were 0, 0.2, 1 and 6 mg/m^3. The effect of chronic exposure of rats to respirable polymeric MDI aerosol was confined to the respiratory tract. The compound-related changes were found in the nasal cavity, the lungs and the mediastinal lymph nodes, and to some degree they were already present after 1 year of exposure. The findings in the present study are, qualitatively and quantitatively, fully in line with the results of the short-term inhalation studies by Reuzel, 1994ab. From the results of the present study it was concluded that the no-adverse-effect level for the toxicity of polymeric MDI was 0.2 mg/m^3. The LOAEL was set on 1.0 mg/m^3. For more detail see below and Section 4.1.2.8.

In both the long-term and subchronic animal studies with polymeric MDI by Reuzel (1994a, 1994b) compound-associated, yellowish particulate material was found in alveolar luminal macrophages. The amount of particulate material accumulated at the level of the alveolar duct, increased with time as well as with level of exposure. Macrophages with yellow pigment were also found in the alveolar interstitium and accumulation of these macrophages also occurred in the mediastinal lymph nodes. This points to transportation of the material from the lungs to the associated lymph nodes.

A short-term inhalation toxicity study of polymeric MDI in rats was designed to investigate both the relationship between acute irritation and alteration of surfactant activity (Pauluhn et al., 1998). The first aspect was addressed by analysis of changes in breathing patterns during an acute inhalation exposure, the second aspect was addressed in a 2-week repeated nose-only

inhalation study with a mean analytical concentration of 1.1, 3.3 and 13.7 mg polymeric MDI/m^3 (6 hours/day, 15 exposures). The results show that rats exposed to 3.3 and 13.7 mg/m³ experienced mild signs of respiratory tract irritation which appear to exacerbate during the course of the study. According to the authors, light and transmission electron microscopy suggest that exposure to 3.3 and 13.7 mg/m³ resulted in an accumulation of refractile, yellowish-brownish material in alveolar macrophages with concomitant activation of type II pneumocytes. The authors suggest that polymeric MDI appears to interact directly with pulmonary surfactant lining fluids, the first line of pulmonary defence. This assumption is further corroborated by increased levels of intracellular phospholipids – evidenced by three independent methods, i.e., polychrome stain, determination of phosphatidylcholine and electron microscopy. Statistically significant changes in the phospholipid content of alveolar macrophages equal to or exceeding 1.1 mg/m³ MDI were found. In the terminal bronchioles a concentration-dependent increase of bromodeoxyuridine-labelled epithelial cells was observed in all polymeric MDI exposure groups. The findings obtained suggest that the interaction of polymeric MDI with surfactant constituents eventually leads to intracellular precipitates originating from precipitated surfactant or surfactant-polymeric MDI complexes. The human significance of the findings is unclear at present. A more elaborate discussion of these findings is to be found in Section 4.1.2.3.1. (Respiratory tract irritation).

In line with Pauluhn et al. (1998), Kilgour et al. (2002) reported an acute inhalation study combined with a subacute inhalation study (28-days) designed to evaluate early changes in the lungs of female Wistar rats resulting from exposure to polymeric MDI.

In the acute inhalation study, groups of 40 female rats were exposed (nose-only) to target concentrations of 0, 10, 30, or 100 mg/m³ polymeric MDI for 6 hours. At 1, 3, 10, or 30 days following exposure, 5 rats from each group were taken for analysis of lung lavage components and 5 for pathological examination. Acute exposures produced clinical signs in all animals that were consistent with exposure to irritant aerosols (abnormal respiratory noise, breathing rate reduced and depth increased, mucous secretions from the nose). An exposure concentration-related body weight loss and increase in lung weight were seen post-exposure, with complete recovery by day 10. Immediately following exposure there were increases in total cells, total protein, alkaline phosphatase, NAG and some indication of increased LDH activity in lung lavage fluid. By day 3 post-exposure, further increases were apparent in total cell counts. LDH activity was elevated in all groups to a greater extent than on day 1 post-exposure, although alkaline phosphatase and NAG activity had returned to control levels. Increases in cell replication became apparent in both the terminal bronchioles and centro-acinar alveolar regions examined, the response being concentration-dependent, correlating with the concentration-dependent bronchiolar hyperplasia seen histologically and type II cell hyperplasia identified by electron microscopy. By day 10 post-exposure, most of the measured parameters had returned to control levels. Cell proliferation was still slightly higher than control levels in the 30 mg/m³ group. At the light microscopy level, macrophage accumulations were still evident in animals exposed to 10 mg/m³ only, epithelialisation of the alveoli was present in animals exposed to 30 and 100 mg/m³ and thickening of the alveolar wall and ducts were evident in animals exposed to all concentrations, although generalised effects had resolved to a large extent. By day 30 post-exposure, lung weights, lung lavage parameters, cell proliferation and ultra structural appearance had returned to normal at all exposure concentrations. Some slight epithelialisation of the alveolar duct and cell exudate in the lumen was still evident at low incidence in the 100 mg/m³ group, but all other effects had recovered. The time course of changes in the lung over the initial days following exposure consisted of a pattern of initial toxicity, rapid and heavy influx of inflammatory cells and soluble markers of inflammation and cell damage, increased lung surfactant, a subsequent recovery and epithelial proliferative phase and, finally, a return to

the normal status quo of the lung. During these stages there was evidence of perturbation of lung surfactant homeostasis, demonstrated by increased amounts of crystalline surfactant and increased number and size of lamellar bodies within type II alveolar cells.

Repeated exposure over 28 days to 1, 4, or 10 mg/m³ polymeric MDI (6 hours/day, 5 days/week, 4 weeks, nose-only, groups of 30 female Wistar rats) produced no clinical signs or body weight changes, but an increase in lung weight was seen in animals exposed to 10 mg/m³ (35%) which resolved following the 30-day recovery period. Other effects seen were again consistent with exposure to irritant aerosols, but were less severe than those seen in the acute study. Analysis of bronchoalveolar lavage fluid showed changes in the majority of parameters at 10 mg/m³. Total cell count was increased statistically significant and this was accounted for by increases in alveolar macrophages, PMNs and lymphocytes/other cell types. At both 4 and 10 mg/m³ polymeric MDI increased numbers of 'foamy' macrophages in lung lavage cell pellet correlated with the increased phospholipid content of the pellet. Changes in lung lavage biochemical parameters (10 mg/m³: moderate increases only in total protein, LDH, alkaline phosphatase and phospholipids, no effect on NAG) and electron microscopic evidence again suggested perturbations in surfactant homeostasis. In alveolar macrophages, minimal to slight increases in lamellar surfactant were with minimal and moderate increases in amorphous surfactant in animals exposed to 10 mg/m³. In the alveolar lumina, compound-related increases in the amount of crystalline and lamellar surfactant were associated with the minimal to moderate increases in cell debris noted in animals exposed to 4 or 10 mg/m³. At 1 mg/m³, there was also some evidence of effect on surfactant homeostasis, with small increases in number and size of type II cell lamellar bodies and similar increases in amorphous, crystalline and lamellar surfactant in the alveolar lumina. Histologically, bronchiolitis and thickening of the central acinar regions was seen at 4 and 10 mg/m³, reflecting changes in cell proliferation in the terminal bronchioles and centro-acinar regions. In animals exposed to 1 mg/m³ polymeric MDI, 1/5 animals showed bronchiolitis. Almost all effects seen had recovered by day 30 post-exposure. Although, after the recovery phase, alveolar macrophages containing a yellow pigment were still present in the interstitium in all animals that had been exposed to 10 mg/m³ polymeric MDI but were absent in animals exposed to 1 or 4 mg/m³ polymeric MDI. In addition, 1/5 animals exposed to 10 mg/m³ polymeric MDI still had bronchiolitis and centro-acinar thickening, but at a reduced severity and distribution. The results are consistent with pulmonary / cellular stress in response to chemically reactive particulates. These findings suggest that an exposure concentration of 1 mg/m³ (duration of exposure 6 hours/day) for 28 days, caused non-specific cell proliferation of Type II pneumocytes.

In summary, according to the authors, exposure of rats to respirable aerosols of polymeric MDI for single acute or repeated subacute exposures resulted in a pattern of lung responses that is entirely consistent with exposure to irritant aerosols.

According to the rapporteur, in the subacute study, 1 mg/m³ is the LOAEL for effects on surfactant homeostasis and (reversible) bronchiolitis (NOAEL< 1 mg/m³), whereas the NOAEL for pneumonitis is less than 10 mg/m³.

A chronic inhalation study (Hoymann et al., 1995) has also been conducted with monomeric 4,4'-MDI. Female Wistar rats were exposed to 0.23, 0.7 or 2.05 mg/m^3 4,4'-MDI aerosols for 17 hours/day, 5 days /week for up to 24 months. 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 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 NOAEL in this study was 0.23 mg/m^3. For more detail see below and Section 4.1.2.8.

It should be noted that the exposure durations differed substantially between the 2 chronic studies and hence the doses received by the lungs of the test animals also differed between the studies.

Under the auspices of the International Isocyanate Institute an expert review has been conducted of the Reuzel et al. (1990) and the Hoymann et al. (1995) bioassays to provide a comparison of dosimetry and pathological responses (III report, 1999; Feron V. et al., 2001). The review has shown very good consistency across the studies with respect to gradation of inhaled dose and the observed histopathological changes. The outcome can be summarised as follows:

Table 4.30 Key studies on repeated dose toxicity: exposure regimen, dose groups, animals

Study	Reuzel et al., 1990			Hoymann et al., 1995		
Test substance	Polymeric MDI			Monomeric MDI		
Colour	Dark brown liquid			Yellow-white		
Atmosphere generation	Condensation aerosol			Nebuliser		
Exposure duration	6 hours/day; 5 days/week			17 hours/day; 5days/week		
Concentration mg/m³	0.19	0.98	6.03	0.23	0.7	2.05
Cumulative concentration mg h/m³	558	2,881	17,730	2,003	6,188	18,120
Particle size MMAD (µm)	0.73			1.1		
GSD	2.5			1.46		
Group size	60			80		
Rat strain	Wistar (Cpb:WU)			Wistar (Crl:[Wi]Br)		
Sex	Males and females			Females		

It should be noted that the highest concentration used in the Reuzel et al. (1990) study represented the Maximum Tolerated Concentration (MTC). This was derived from results of a number of preliminary sub-chronic inhalation studies that demonstrated that at a higher concentration there was an increased risk of mortality due to severe respiratory tract toxicity.

Table 4.31 Key studies on repeated dose toxicity: MDI-induced lesions observed (incidences given in percent)

Study	Reuzel et al., 1990				Hoymann et al., 1995			
Concentration (mg/m³)	0	0.19	0.98	6.03	0	0.23	0.7	2.05
Cumulative concentration (mg h/m³)	0	558	2,881	17,730	0	2,033	6,188	18,120
Adenoma, bronchiolo-alveolar	0	0	0	3.4	0	0	0	1.3
Hyperplasia, bronchiolo-alveolar	10	15	41.7	100	18.6	20	33.8	66.3
- alveolar type	2.5	8.3	13.3	50.8	11.9	13.8	16.3	36.3
- defined as pre-neoplastic	0	0	0	13.6	0	0	2.6	8.8
- bronchiolar type	2.5	1.7	20	100	0	10	15	52.5

Table 4.31 continued overleaf

Table 4.31 continued Key studies on repeated dose toxicity: MDI-induced lesions observed (incidences given in percent)

Study	Reuzel et al., 1990				Hoymann et al., 1995			
Interstitial fibrosis	0	0	31.7	100	0	78.8	96.3	100
Increased mineralised deposits (calcified deposits + osseous metaplasia)	6.3	6.7	15	67.8	10.2	13.8	10.1	35.1
Mononuclear cell infiltration	30	55	51.7	88.1	45.8	45	62.5	87.5
Particle-laden macrophages	0	51.7	93.3	100	0	66.3	87.5	100

Female animals

In both studies qualitatively similar responses were observed, i.e. adenoma, bronchiolo-alveolar hyperplasia and lung fibrosis. In the Reuzel et al. (1990) study two pulmonary adenomas were found in the high dose level group whilst in the Hoymann et al. (1995) study one such tumour was found at a similar cumulative exposure level. Pre-neoplastic lesions were found in both top dose groups (17,730 – 18,120 mg h/m³) and in the mid-dose Hoymann et al. group (6,188 mg h/m³). Tumours as well as pre-neoplastic lesions had a late onset, since in the Reuzel et al. study only a few animals died prematurely from a tumour or pre-neoplastic lesion. While this late onset is also inferred from the Hoymann et al. study, the mean survival time of rats in this latter study was poor across all groups in general due to an extraordinarily high rate of pituitary neoplasms which was not related to treatment. In the MDI aerosol exposure groups of both studies alveolar macrophages contained material indicating the high respirability of test aerosol particles.

Male animals

Although male animals of the Reuzel et al. study were not included in the study comparison, a review of the slides with lung tumours indicated that the MDI induced lesions in males were the same as in females: bronchiolo-alveolar adenoma, bronchiolo-alveolar hyperplasia (reported as alveolar duct epithelialisation and alveolar bronchiolisation (Reuzel et al., 1994a); interstitial fibrosis; mineralised deposits.

Six males of the top dose group were found with primary lung tumours, of which five were single adenomas and one was an adenocarcinoma (this tumour type was not observed in females). All these neoplasms occurred in animals surviving to termination of the study. Bronchiolo-alveolar hyperplasia of the alveolar type (previously reported as 'alveolar bronchiolisation' in Reuzel et al., 1994a) and including pre-neoplastic lesions) was increased in the top dose group.

There was no evidence of any extra pulmonary neoplastic or non-neoplastic lesions except for irritant related portal of entry effects in the upper respiratory tract.

4.1.2.6.2 Studies in humans

The long-term effect of isocyanates on the respiratory system was studied in 318 workers employed in 2 factories using MDI, and some TDI, for the production of polyurethane foams (Pham et al., 1978). Atmospheric MDI levels at Plant A were consistently lower than the maximum acceptable concentration (MAC value) of 0.2 mg/m^3, whereas at Plant B peaks of up to 0.87 mg MDI/m^3 were sometimes found at the foam injection workplaces. The atmospheric MDI levels were determined by the technical departments of the French National Research and

Safety Institute, using the Meddle et al. colorimetry method with a Technicon Air Monitor IV. Other chemicals employed included polyols, amines, additives such as silicones and pigments, and expanding agents. Of the 318 workers, 277 were employed at Plant A and 41 at Plant B. They were divided into three groups based on exposure. Group I contained those not exposed to occupational hazard (83); Group II was those indirectly exposed to risks associated with foam plastics manufacture (117); Group III was those directly exposed to risks due to foam plastics manufacture (118). There was examination of a random 1 in 5 of each group by questionnaire, clinical examination, and pulmonary function tests.

There were no significant differences between the three groups, for men or women, with regard to age, height or smoking habit. Group II and III reported slightly more symptoms which mostly indicated bronchitis. The number of men having a VC or FEV1 below 90% predicted, or showing a fall in FEV1 after acetylcholine challenge was significantly greater in Group III than in group I. For women the frequency of the transfer coefficient Kco (diffusion constant) values < 4 was greater in Group III than Group II. Men in Group II and III had significantly lower VC and Tco (transfer factor) than those in Group I. In Group III the mean values of VC as a percentage of predicted, and of Kco, in men who had been working in exposed areas for more than 60 months were significantly lower than in men with a shorter exposure.

It was concluded that long-term exposure to isocyanates tends to cause restriction of pulmonary function and a decline in Tco, and the possibility of fibrosis after long exposure was suggested.

Continuation of this work in a 5-year longitudinal study is reported in Pham et al. (1988).

After 5 years only half the initial cohort was still active (114 males and 45 females). As there was a large reduction in the number of females, only the results of the males were reported. For the longitudinal analysis the workers were classified as:

(a) unexposed on both occasions
(b) indirectly exposed on both occasions
(c) directly exposed on both occasions
(d) exposed on the first occasion but removed from contact with MDI before the second occasion

The number of workers with asthma or chronic bronchitis increased over 5 years but in all groups. Pulmonary function indices of the second study confirmed the lower values of exposed workers on the first occasion. The results were normal for group (d). The decline in VC and FEV1 was not significantly different between the groups, but a significantly greater decline in Dlco was found for those with persisting exposure.

It was concluded that chronic exposure to even low levels of isocyanates involved a respiratory risk.

A case report describes fibrosing alveolitis in a man following exposure to MDI (Friedman, 1982, abstract only). A 46-year old male manufacturing engineer received prolonged exposure to MDI several times greater than the OSHA (Occupational Safety and Health Administration) limits. Symptoms of hypersensitivity pneumonitis and pleuritis developed, which did not clear over the following three years. Pulmonary functions were initially unremarkable but have changed over 3 years to be consistent with slowly progressive fibrosing alveolitis, and transbronchial lung biopsies have confirmed this.

One hundred and seven subjects from a polyurethane plastic manufacturing plant were followed over a five-year period with measurements of the FEV_1, and questionnaires on respiratory

symptoms and smoking habits (Musk et al., 1982). Environmental concentrations of TDI and MDI were extensively monitored by the Marcali method to provide estimates of the upper-limits of exposure of the subjects. Ventilatory function was examined at the beginning and the end of a workshift to assess whether any acute change was occurring. The measurements were also repeated before and after a vacation to detect any short-term improvement in function that may reflect recovery from exposure-induced bronchoconstriction. Subjects were also examined over a five-year interval to measure long-term decrement in pulmonary function. Over the five years of the study, 2,573 environmental samples were collected by the plant industrial hygiene department using handheld samplers in the breathing zone of subjects employed in pouring the urethane plastic. Sampling thus emphasised the areas in the plant where the highest exposures were encountered. During each day of the survey of lung function, further environmental measurements at sites selected to show highest concentrations of TDI and MDI were again made concurrently by the plant industrial hygiene department and the investigators. Sampling time was from 20 to 60 minutes and analysis was done by the method of Marcali which was adapted for MDI. Exposure category for each subject included in the study was determined from all the environmental measurements made over the past five years and from the occupational history. Environmental measurements performed at the time of the surveys of respiratory function revealed very small concentrations of isocyanates. Ninety percent of all measurements of MDI taken over the four years prior to the follow-up study by the plant industrial hygiene department contained less than 0.022 mg/m³ in plant 1 and 0.012 mg/m³ in plant 2. The geometric mean MDI concentrations were 0.006 mg/m³ in plant 1 and 0.003 mg/m³ in plant 2. Current mean levels of the FEV_1 in this population were higher than those predicted for healthy subjects. The five-year change in FEV_1 did not exceed that expected from ageing. No acute change in FEV_1 could be demonstrated over the course of a Monday either before or after a two-week vacation. No improvement in ventilatory function was observed over the vacation period. The presence of cough or sputum was related to smoking but was not related to isocyanate exposure. The results indicated that exposure of workers to extremely low levels of isocyanates (time-weighted average concentrations of the order of 0.01 mg/m^3) was not associated with chronic respiratory symptoms or effects on ventilatory capacity.

A cross-sectional study of workers exposed to an MDI-based insulating foam during refrigerator manufacture was conducted by Saia et al. (1976) using the CECA (Communauté Européenne du Charbon et de l'Acier) questionnaire for chronic bronchitis and emphysema, physical examination of the chest and spirographic tests (Expirograph – Godart), determination of VC, FEV_1, before and 5 minutes after administration of a bronchodilatator. A complete examination was made of 180 workers (94 furnace workers, 32 injectors, and 54 assembly line workers), all working during the investigation and all without acute respiratory disease. Chronic bronchitis and sputum breathlessness increased with age and length of exposure. There was a significant difference between smokers and non-smokers with regard to simple bronchitis. The furnace men were more prone to both syndromes than the injectors, or assembly line workers. This was presumed to be due to greater exposure. Those positive for bronchial asthma totalled 6.7% of the whole population.

The effects of exposure on spirometry in this population was assessed by Fabbri et al. (1976). There was a decrease of >10% in VC in 51% of the subjects, and of >20% in 16%. The corresponding figures of the FEV_1 were 59%, and 20%, respectively. There were very few cases of broncho-obstructions and the degree of reversibility was small since only 7% of the subjects improved after inhaling Orciprenaline. There was a relationship between the questionnaire-based syndromes of "Sputum Breathlessness" and "Simple Bronchitis" and the prevalence of reduced VC and FEV_1. For the analysis of the effects of exposure the only workers included were those (160) who did not have previous exposure to respiratory irritants. When the workers were

divided into 3 groups having exposures of 0-4, 4-8, 8-12 years, respectively, it was found that there was a decline in VC and FEV_1 after the first 4 years. The indices were more or less constant thereafter. The prevalences of significant changes in the indices increased after 4 years and then stabilised. Amongst the performed jobs the highest prevalence of spirographic impairments and subjective respiratory symptoms were found in the furnace workers. There was, however, no relationship between job and degree of impairment, even when taking into account the length of exposure.

In a retrospective analysis (1964-1979), a group of 109 workers involved in the production of monomers and polymers of MDI was compared with a group of 83 workers on other sections of the same chemical works without known exposure to materials with corrosive effects on the respiratory system (Diller and Derbert, 1983). Both groups showed a decrease in forced vital capacity (FVC) with increasing age, which was significantly greater than in the Commission of European Communities Tables of Values. Workers employed for more than 5 years in MDI production and other parts of the chemical works had significantly lower FVC than workers with less than 5 years employment in the works. In a 2-year study (1976 and 1978) with 15 control and 88 MDI-exposed workers, the average decrease in FVC in workers with moderate exposure to MDI was twice as great as that in workers with little or no exposure. In contrast, the decrease in FEV_1 was about 4 times greater in workers with no exposure than in those with slight exposure (4.66 against 0.94%) and slightly greater than in those with moderate exposure (3.87%). Lung-function analysis differences between MDI-exposed and control workers were not significant. Eight of the 109 MDI workers had chronic obstructive bronchial diseases and 3 had contact dermatitis associated with chlorophenylisocyanate. None of the control subjects had bronchial disease or dermatitis. Absence due to sickness was less in the MDI than in the control group. Possible hypersensitivity of the respiratory system to MDI was the cause of transfer to other work in 11 (3.8%) workers in the 14 years since the start of the works. Concentrations of isocyanates in the air were in general very much less than the MAK value (0.2 mg/m³) but did occasionally reach or exceed this value (no more data available).

Sulotto et al. (1990) studied 27 polyurethane foam workers exposed to MDI only at low concentrations (ranging from 0.005 to 0.01 mg/m³) and 27 clerks from the same factory (producing finished parts for the car industry) matched by age. Environmental analyses to assess MDI concentration were carried out by continuous tape monitoring (7005 FR Rankon Analyzer) during the same period when functional tests were performed. The exposure values ranged from 0.005 to 0.01 mg MDI/m³. Respiratory function tests were performed with a Vicatest dry spirometer, on a Monday and Friday of the same week at shift onset, 4 hours later and at shift end. The subjects under study were asymptomatic for asthma. The two groups had quite similar spirometric values with minimal functional impairment. A statistical analysis was carried out in order to take into account both occupational exposure and smoking habits. No significant differences between the two groups were observed in the respiratory parameter trend during both the Monday and Friday work shift. Nor were differences observed within the two groups when Friday's and Monday's results were compared. No significant differences between the two groups were found in paired comparisons between Friday and Monday for respiratory parameters. FEV_1 and FEF_{25-75} reduction present on Friday, when compared to Monday, was related to smoking and not to occupational exposure. The authors concluded that their findings showed no short-term respiratory changes in subjects exposed to low MDI concentrations.

Jang et al. (2000) investigated the prevalence of airway hyper responsiveness induced by MDI and TDI at a petrochemical industry complex in Korea. A total of 64 workers aged 28-48 years were studied, consisting of 44 workers exposed to TDI and 20 workers exposed to MDI. Exposed workers were currently working and were selected randomly from 1 TDI manufacturer

and 1 MDI factory. The control group, 27 healthy subjects (23 men, 4 women), average age 35.9 years, were recruited from a workshop and field staff with no exposure to known asthma inducing agents. Ambient air concentrations of TDI and MDI were measured during manufacture at the workplace. A total of 60 personal breathing zone samples were collected according to Streicher et al. (1996), sampling time being 30-60 minutes. Mean ambient air concentrations of TDI and MDI were under the Threshold Limit Values-Time Weighted Average (based on Threshold Limit Values and Biological Exposure Indices, ACGIH 1995). For MDI the mean ambient air concentration was 0.0013 mg/m³ with a maximum of 0.0064 mg/m³; for TDI the mean was 0.0174 mg/m³ with a maximum of 0.0429 mg/m³. Questionnaires, allergic skin tests, and non-specific airway hyper responsiveness (AHR) were studied. Questionnaires included questions about symptoms of cough, wheezing, chest tightness, dyspnea, rhinorrhea, sneezing, itching, stuffiness, tearing, urticaria, sore throat, and "exacerbating time" (i.e. the time at which these symptoms occurred). Methacholine challenge tests were done. The degree of bronchial responsiveness (Brindex) was defined as log (% fall in FEV_1)/log (last concentration of methacholine + 10). Prevalence of AHR (PC20 FEV_1 < 16.0 mg/ml methacholine) was higher in MDI-exposed workers than in TDI-exposed workers [4/20 (20%) versus 2/42 (4.7%), $P<0.05$]. Twenty-three workers (36% of all subjects) had respiratory symptoms. MDI-exposed workers, in comparison with control subjects, had higher Brindex (0.73 ± 0.04 versus. 0.62 ± 0.02, $P<0.005$). Workers exposed to TDI or MDI who had respiratory symptoms (n = 23), in comparison to workers exposed to TDI or MDI without respiratory symptoms (n = 41), had significantly higher Brindex (0.82 ± 0.06 versus. 0.60 ± 0.02, $P<0.05$). FEV_1 was significantly negatively correlated with Brindex ($r = -0.253$, $P<0.05$). Brindex was not correlated with atopy, smoking status, and exposure duration. According to the authors, the findings suggest that workers exposed to MDI are at a higher risk of asthma in comparison with TDI-exposed workers and control subjects at a petrochemical plant in Korea.

4.1.2.6.3 Summary

As respiratory tract effects are characteristic for the toxicity of isocyanates (Karol, 1986), it was not surprising to find the respiratory tract to be the target organ system of respirable polymeric MDI aerosol.

In both the long-term and subchronic animal studies with polymeric MDI by Reuzel (1994a, 1994b) compound-associated, yellowish particulate material was found in alveolar luminal macrophages.

In humans, some, but not all, epidemiological studies have found long-term decreases in ventilatory function and respiratory symptoms, in workers exposed to MDI even below current occupational standards.

For short-term toxicity (in rats), the most reliable LOAEL found for increased lung weights is 2 mg/m^3, the most reliable NOAEL being 1.4 mg/m³. However, in a more recent mechanistic study (also short-term toxicity in rats) a LOEL of 1.1 mg/m³ was found for changes in the phospholipid content of alveolar macrophages and non-specific cell proliferation of Type II pneumocytes. These findings are consistent with the LOAEL (subacute) of 1 mg/m³ found for effects on the surfactant homeostasis by Kilgour et al. (2002). In an acute inhalation study (see Section 4.1.2.2) a LOAEL of 0.7 mg/m³ was found for transient dysfunction of the pulmonary epithelial barrier, related to a dysfunction of pulmonary surfactant. Subsequently, the same author estimated a likely NOAEL of 0.5 mg/m³ for respiratory tract irritation. Although

these transient alternations are possibly of no major concern, the rapporteur is of the opinion that it is appropriate to use this estimated NOAEL of 0.5 mg/m³.[12]

The most reliable NOAEL for chronic toxicity (in rats) seems to be 0.2 mg/m^3. The most reliable LOAEL is 1 mg/m^3.

For further risk characterisation, the NOAEL = 0.2 mg/m³ (Reuzel et al., 1990, 1994a) will be used for long-term inhalation exposure (workers), the NOAEL = 0.5 mg/m³ (Pauluhn, 2002b) will be used for short-term inhalation exposure (consumers).

Table 4.32 Summary overview of studies on repeated dose toxicity

Substance	Species	Method	Endpoint	Reference	Reliability[1]
Monomeric 4,4'-MDI	Rat	Subchronic inhalation study 90 days	NOAEL 0.3 mg/m^3 LOAEL 1 mg/m^3	Heinrich et al., 1991	4
Monomeric 4,4'-MDI	Rat	Chronic inhalation study 2 years	NOAEL 0.23 mg/m^3	Hoymann et al., 1995	2
Polymeric MDI	Rat	Subacute inhalation study 14 days	NOAEL >26.8 mg/m^3	Wazeter, 1964e	3
Polymeric MDI	Rat	Subacute inhalation study 28 days	NOAEL >2.9 mg/m^3	Wazeter, 1964f	3
Polymeric MDI	Rat	Subacute inhalation study 14 days	LOAEL 2 mg/m^3	Reuzel, 1985a	2
Polymeric MDI	Rat	Subacute inhalation study 14 days	No LOAEL	Reuzel, 1985b	3
Polymeric MDI	Rat	Subchronic inhalation study 13 weeks	No clear NOAEL	Reuzel et al., 1985b	2
Polymeric MDI	Rat	Subchronic inhalation study 13 weeks	NOAEL <4 mg/m^3	Reuzel et al., 1986	2
Polymeric MDI	Rat	Acute, subacute, subchronic inhalation studies	NOAEL 1.4 mg/m³, (the actual NAEL < but probably very close to 4.1 mg/m³)	Reuzel et al., 1994b	2

Table 4.32 continued overleaf

Table 4.32 continued Summary overview of studies on repeated dose toxicity

Substance	Species	Method	Endpoint	Reference	Reliability[1]
Polymeric MDI	Rat	Chronic inhalation study 2 years	NOAEL 0.2 mg/m^3 LOAEL 1 mg/m^3	Reuzel et al., 1990, 1994a	1 2
Polymeric MDI	Rat	Short-term inhalation study	Interaction with pulmonary surfactant: non-specific cell proliferation of Type II pneumocytes LOEL: 1.1 mg/m³	Pauluhn et al., 1998	2
Polymeric MDI	Rat	Subacute inhalation study	Effect on the surfactant homeostasis and (reversible) bronchiolitis LOAEL: 1 mg/m³	Kilgour et al., 2002	3
MDI no more data	Human	questionnaire, clinical exam., pulm. function tests	Reduction of pulmonary function, possibility of fibrosis, decline in Tco	Pham et al., 1978	3
MDI no more data	Human	questionnaire, clinical exam., pulm. function tests	Idem	Pham et al., 1988	3
MDI no more data	Human	questionnaire, clinical exam., pulm. function tests	Hypersensitivity pneumonoitis, pleuritis, progressive fibrosing alveolitis	Friedman, 1982	4

Table 4.32 continued overleaf

Table 4.32 continued Summary overview of studies on repeated dose toxicity

Substance	Species	Method	Endpoint	Reference	Reliability[1]
MDI no more data	Human	questionnaire, pulm. function tests	Low levels: no association with chronic respir. Symptoms or effects on ventilatory capacity	Musk et al., 1982	3
MDI no more data	Human	CECA questionnaire	6.7% of the whole population were positive for bronchial asthma	Saia et al., 1976	3
MDI no more data	Human	pulm. function tests	Reduction in VC and FEV_1	Fabbri et al., 1976	4
MDI no more data	Human	pulm. function tests	Decrease of FVC	Diller and Derbert 1983	3
MDI no more data	Human	pulm. function tests	No short-term respiratory changes in subjects exposed to low MDI concentrations	Sulotto et al., 1990	3
MDI No more data	Human	questionnaire, pulm. function tests	MDI-workers at higher risk of asthma in comparison with TDI-workers and controls.	Jang et al., 2000	3

1 Reliability key: 1 = method and description are in accordance with test guidelines
2 = falling short of highest standards concerning aspects of protocol or reporting
3 = method or report not in accordance with test guidelines
4 = minimal description of method and report

Further results of effects on the skin and the respiratory tract are presented in the sensitisation studies and respiratory tract irritation studies under Section 4.1.2.3 and 4.1.2.5.

4.1.2.7 Mutagenicity

4.1.2.7.1 *In vitro* studies

Bacterial studies

The application of the Ames test, or a modified Ames test, to MDI dissolved in dimethyl sulphoxide (DMSO) gave a variety of results (Herbold 1980a and 1980b; Herbold 1996a, 1996b and 1996c; Andersen et al., 1980; Shimizu et al., 1985; Zeiger et al., 1987; Woolrich, 1982). In general the *Salmonella typhimurium* strains TA1535, TA1537 and TA1538 gave negative results with, or without, metabolic activation. Negative results were also obtained from TA98 and TA100 without activation. Whilst negative results were obtained from TA98 and TA100 with activation, positive results were more usual.

Herbold (1980a and 1980b) found for monomeric 4,4'-MDI an effect dose of 20µg/plate in TA100 with S9mix, an effect dose of 100 µg/plate in TA98 with S9 mix; for polymeric MDI an effect dose of 125 µg/plate was found in TA100 with S9mix. MDI was considered to be a weak but unequivocal mutagen. In these studies, Endoxan and trypaflavine were used as positive control substances, giving both a marked mutagenic effect.

Herbold (1996a) also assessed 2,4'-MDI in the *Salmonella typhimurium* TA1535, TA1537, TA100 and TA98 strains using DMSO as solvent. 2,4'-MDI was negative in all strains in the absence or presence of metabolic activation. In a first addendum of this report, Herbold (1996c) found evidence of weak mutagenic activity of 2,4'-MDI in DMSO and with a 30% S9mix. The lowest effective dose was 15µg/plate for the TA98 strain and 30µg/plate for the TA1538 strain. In these studies, sodium azide, nitrofurantoin, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene were used as positive control substances, giving a marked mutagenic effect.

Generic MDI was also assessed by Herbold (1996b) using DMSO as a solvent. MDI was found positive in the strains TA100 and TA98 but only in the presence of metabolic activation. In this study, nitrofurantoin, cumene hydroperoxide, 4-nitro-1,2-phenylene diamine and 2-aminoanthracene were used as positive control substances, all giving a marked mutagenic effect.

The mutagenic effect of monomeric 4,4'-MDI, dissolved in DMSO, was also examined by Andersen et al. (1980). MDI was found mutagenic in TA100 after metabolic activation with an effect dose of 100 µg/plate. 4,4'-MDA was used as positive control substance, giving a marked mutagenic effect.

In another well-conducted Ames test by Herbold (1980c), monomeric 4,4'-MDI was tested in acetone as the solvent. In this study, monomeric 4,4'-MDI was found to be a weak but unequivocal mutagen in TA100 with S9mix. The effect dose was set on 20 µg/plate. Precipitation of MDI occurred at the dose of 2,500 µg per plate. In this study, they made use of Endoxan and 2-aminoanthracene as positive control substances, giving both a marked mutagenic effect.

When anhydrous ethylene glycol dimethylether (EGDE) was used as the solvent in the Ames test, both monomeric 4,4'-MDI and polymeric MDI gave negative results with TA100 with, or without activation (Herbold, Report n° 19561 and 19570, 1990a and b). At all doses (at 150 µg per plate and above), MDI had a bacteriotoxic effect, so the range could only be used to a limited extent up to 2400 µg per plate. MDI precipitation occurred at the dose of 600µg per plate and

above. No biologically relevant increase in the mutant count, in comparison with the negative controls was observed. The positive controls, nitrofurantoin and 2-aminoanthracene, had a marked mutagenic effect. MDI was considered as non-mutagenic in S. typhimurium TA100.

It appears that solvent effects contributed to the positive results. MDI is not stable in DMSO; many products are generated within minutes (Gahlmann et al., 1993). Thus it seems possible that positive test results are caused by the degradation products of MDI in DMSO, rather than by MDI itself. One of the degradation products of MDI is the amine 4,4'-methylenedianiline (MDA), which is known to be genotoxic.

Recent publications have expanded on this issue. Herbold et al. (1998) and Seel et al. (1999) determined the stability of monomeric 4,4'-MDI in DMSO and EGDE. IR spectroscopy was used to quantify the stability of the isocyanate function, and HPLC to follow the disappearance of MDI and formation of breakdown products including 4,4'-MDA. The mutagenic activity of solutions of MDI in DMSO and EDGE was assessed in 4 or 5 *Salmonella typhimurium* tester strains (TA 98, TA 100, TA 1535, TA 1537, TA 1538). These latter investigations were conducted both in the absence and in the presence of a metabolic activating system (S9 mix) and they included a number of isomers (2,4'-MDI; 4,4'-MDI; mixed MDI isomers, comprising 4,4'-, 2,4'- and 2,2'-MDI; polymeric MDI). As positive control substances, cyclophosphamide, trypaflavine and 2-aminoanthracene were used, giving a marked mutagenic effect.

Results of the IR analyses showed a very rapid loss of NCO functionality when 4,4'-MDI dissolved in 'dry' DMSO (0.03-0.04% water), with less than 40% of the initial amount remaining after 15 minutes, and almost complete breakdown occurring within 2 hours. This pattern was confirmed by HPLC, which also detected concurrent formation of breakdown products, including the mutagen 4,4'-MDA (3 – 9% w/v). In contrast, solutions of MDI in 'dry' EDGE (0.02% water) were quite stable, with a relatively small loss of diisocyanate from the incubation and no detectable formation of MDA. Stability was only marginally affected by the addition of water, revealing significant differences in chemical kinetics when compared with that seen in DMSO.

In the mutation tests, no activity was detected with any MDI isomer in the absence of S9 irrespective of whether DMSO or EGDE was used as vehicle, nor was any mutagenicity found when EGDE was used in the presence of S9. In contrast, consistently positive results were obtained in TA 98 for all MDI isomers when dissolved in DMSO and co-incubated in the presence of S9 fraction, and a generally similar response was seen with TA 100 although 2,4'-MDI was inactive in this tester strain. The latter isomer was, however, mutagenic towards TA 1538 when DMSO was used as vehicle, in the presence of S9.

The investigators employed a range of dilutions of MDI in DMSO, starting at 4-8 µg/plate up to a maximum of several thousand µg/plate (amounts that were invariably toxic to *Salmonella typhimurium*). A threshold, below which no mutagenic activity was detectable, can be derived from this work. For tester strain TA 100, this 'threshold of detection' was at or above 20, 75 or 100 µg/plate for monomeric 4,4'-MDI, mixed MDI or polymeric MDI, respectively, when DMSO was used as vehicle. The equivalent concentrations for TA 98 were 100, 75 or 500 µg/plate (concentrations refer to the initial concentration of MDI present at the start of the incubation). Comparable data exist on the mutagenicity threshold for MDA in these same test organisms, with TA 100 responding to 20µg/plate and TA 98 to 200 µg/plate and above (MDA IUCLID HEDSET). The findings indicate that *Salmonella typhimurium* strains TA 98 and TA 100 can detect formation of reasonably small amounts of MDA *in vitro*. The absence of any MDI-derived mutagenic activity when EGDE was used as solvent is therefore consistent with negligible conversion to MDA under these experimental conditions.

The studies of Herbold et al. (1998) demonstrate that MDI is not stable in DMSO, and as a consequence breakdown products, in particular MDA, are formed rapidly. MDA is a known genotoxin, and produces a mutagenic response in *Salmonella typhimurium* tester strains TA 98 and TA 100, but only in the presence of a hepatic metabolic activating system. This profile is entirely consistent with that for MDI dissolved in DMSO, suggesting strongly the involvement of a common mutagenic component (MDA). In contrast, MDI is stable in EDGE and no mutagenic activity is detectable in these same microbial systems. These observations help explain the underlying inconsistencies present in the *in vitro* genotoxicity data base for MDI. They also clearly demonstrate that no mutagenic activity is detectable under conditions where MDA is not produced.

It is evident from the results of studies described above that the solvent used for solubilisation of MDI in genotoxicity assays is crucial with respect to a valid and representative evaluation of MDI in such assays. There is adequate evidence to demonstrate that use of EDGE as solvent is appropriate whereas DMSO can give rise to false positives due to solvent-catalysed conversion to MDA. The latter solvent should not be used for MDI in these assays and studies showing positive results as a result of the use of this solvent should be dismissed, provided it can be excluded that rapid catalysis of MDI to MDA occurs *in vivo*. The latter remains to be demonstrated by appropriate toxicokinetic studies.

Mammalian cell studies

Gene mutation assays

Well-conducted Mouse Lymphoma L5178Y specific locus mutation studies are available for both monomeric and polymeric MDI dissolved in DMSO (McGregor et al., 1981a and 1981b). Mutation from $TK^{+/-}$ to $TK^{-/-}$ was measured using a modification of the method of Clive et al. (1972; 1977). The experiments were carried out over the concentration range 2.5 µg/ml to 250 µg/ml. The criterion used to describe a positive result in these tests was a doubling of mutation frequency over the solvent treated value.

In conclusion, there is some evidence that monomeric 4,4'-MDI, dissolved in DMSO, showed mutagenic activity in this mutation test, in the presence of S9mix, but only under conditions of very high toxicity of the compound: ≥ 200 µg/ml (95% toxicity). Precipitation of MDI started at 100 µg/ml.

Polymeric MDI showed no evidence of mutagenic activity in the mouse lymphoma forward mutation assay. Polymeric MDI was cytotoxic at 106 µg/ml and above.

Sister chromatid exchange

In a cytogenetics assay, human whole-blood lymphocytes were exposed to 4,4'-MDI (technical grade) dissolved in acetone (Mäki-Paakkanen et al., 1987). MDI induced chromosome aberrations at all doses tested (0.54-4.30 µl/ml) after a 24-hour treatment in the absence of metabolic activation. In the presence of rat liver S9mix (1.5-hou treatment), only at the highest dose (4.30 µl/ml) were aberrations significantly increased. MDI also marginally increased sister-chromatid exchanges at the highest dose available (2.17 µl/ml) with and without (48-hour treatment) S9mix. On addition to culture medium, MDI formed polymer-like fibres, which were seen as small particles on the microscopic slides, at all doses. At the high doses the presence of these polymers made metaphase analysis impossible. Consequently, toxic doses could not be determined accurately.

One possibility is that the observed cytogenetic effects resulted from the indirect effects mediated by reactive radicals possibly created in the reaction of MDI with water or through uptake of the polymer particles by phagocytising leukocytes. The lack of a clear dose-response in the induction of chromosome damage may be related to the solubility of MDI or the reactive degradation products (e.g. MDA).

Cell transformation

Two studies by Poole et al. (1980a and 1980b) have been summarised in this section.

BHK21 C13 hamster cells were used in the modified cell transformations test described by Styles, 1977.

Cells treated with monomeric 4,4'-MDI, in the presence of S9mix caused at LC_{50} 10.1, 4.2 and 5.5 fold increases in transformation frequency as compared to the negative control (DMSO); polymeric MDI treated cells caused 47.2, 13.2, 1.8 and 10.2 fold increases. In the absence of S9mix 1.1 and 9 fold increases were observed for monomeric 4,4'-MDI; 3.1, 5.6 and 13.5 fold increases for polymeric MDI. These significant increases in the transformation frequencies at the LC_{50} were also accompanied by increases in absolute numbers of transformed colonies in cells treated with both monomeric and polymeric MDI. The results of these studies indicate that MDI is a potential cell-transforming agent. The MDI was dissolved in DMSO.

Other Mammalian cell studies

In a report by Vock et al. (1998), the induction of DNA double-strand breaks by MDI in cultured human epithelial lung cells was investigated to find out whether MDI can induce DNA double-strand breaks by interstrand DNA cross-link formation or whether double-strand breaks are the result of cell death. Cultured human lung epithelial cells (A549) were treated with monomeric 4,4'-MDI, MDA, melphalan (positive control for DNA cross-link) and Triton X-100 (positive control for cell death); all chemicals were dissolved in ethylene glycol dimethyl ether which was added to a cell monolayer covered with phosphate-buffered saline. After 2 hours, the treatment solution was exchanged against medium, and 8, 24 and 72 hours after treatment initiation, the induction of DNA double-strand breaks was assessed by pulsed-field gel electrophoresis. At the same time, the viability was determined with the MTT test (intracellular reduction of the tetrazolium dye MTT). The concentration of MDI required to reduce viability to 50% of control (LC_{50}) was about 200µM at all points investigated. At the 8-hour time point, double-strand breaks were induced only at ≥100 µM and the FAR values (fraction of DNA activity released) increased with time. The dose-response curves for viability and FAR values showed a mirror image, i.e. DNA double-strand break formation was observed only in connection with a reduction in cell viability. MDI induced DNA fragments of a size of 2-3Mbp after 8 hours. Later, fragments became smaller (≤1 Mbp). Treatment of the cells with 30 to 100 µM MDI resulted in irregular clumping of chromatin. The authors conclude that the data give no evidence that MDI induces double-strand breaks by a genotoxic mechanism based on its theoretical DNA-DNA cross-linking ability. It induced cell death by way of necrosis and not apoptosis.

As in a previous study (Zhong and Siegel, 2000: see Section 4.1.2.7.2) the results from *in vitro* exposure of Chinese hamster lung fibroblasts (V79) to MDA, cysteine and glutathione conjugates of MDI (BisCYS-MDI and BisGS-MDI) suggested the genotoxic potential of possible MDI metabolites, Zhong et al. (2001) wanted to distinguish the mechanism of micronuclei (MN) induction following exposure to MDA, BisGS-MDI, and BisCYS-MDI in cytokinesis-blocked V79 cell cultures using the anti-kinetochore immunofluorescence antibody

assay. MDA induced MN in V79 cells in a concentration-related manner, increasing MN 3 to 5 times over the DMSO vehicle control, and 85% of MDA-induced MN were negative with respect to anti-kinetochore antibody binding (KC^-). According to the authors, this is consistent with an interaction between MDA and DNA resulting in chromosome breakage. However, it was observed that treatment of V79 cells with BisGS-MDI and BisCYS-MDI produced a significant increase in the frequency of MN and that the mechanism of MN induction was different from that of MDA. A high percentage of KC^+ was displayed in binucleated cells. According to the authors, these results suggest that the mechanism of MN formation induced by BisGS-MDI and BisCYS-MDI is mediated through disruption and/or by affecting the function of the mitotic spindle. These results are consistent with the findings of Vock et al., 1998). According to the authors, the S-linked conjugates of MDI are aneugens, but the exact mechanism by which they induce aneuploidy is not yet known. The formation of thiocarbamates could be important in the development of isocyanate-induced diseases.

Although there are in this well-conducted study clear indications that MDA induced predominantly chromosome breakage whereas the glutathione and cysteine MDI conjugates induced a dose-dependent aneuploidy, there is a need for additional clarification. According to the III, there is concern that the conjugates synthesised are not of the purity that the authors report. The III have an ongoing program on the synthesis and characterisation of the mono- and bis-glutathione conjugates of MDI, and will repeat the Zhong et al. (2001) study with the appropriate synthesised conjugates.

4.1.2.7.2 *In vivo* studies

Somatic cells

Micronucleus assays

In agreement with OECD Guideline n° 474, groups of 6 mice (10-week-old male ICR mice) were used in the Micronucleus assay by JETOC (1982). The monomeric 4,4'-MDI was dissolved in dry DMSO and this solution was suspended in corn oil. This was administered once by intraperitoneal injection of 10 g/kg bw. The dose levels were 32, 80 and 200 mg MDI/kg (determined from preliminary range finding studies). As the positive control, triethylenemelamine (TEM), dissolved in distilled water, was used. The mice were killed 24 hours after administration. Erythrocytes, 2,000 per mouse, were observed for the incidences of polychromatic erythrocytes (PCE) and normochromatic erythrocytes (NCE) with micronuclei. The presence of effect was suggested by the DMSO/corn oil control giving a reduction in PCE, and an increase in erythrocytes with micronuclei, compared with the distilled water group. In negative controls, the group treated with distilled water and that treated with 4% DMSO-contained corn oil, the incidence of PCE in the latter decreased to approximately 75% of that in the former group and the number of erythrocytes with micronuclei increased 3-fold, suggesting the presence of effect. No significant difference was detected by statistical test. In the positive control using TEM, the incidence of PCE decreased to 55% of that in the negative control (treated with distilled water) and suggested inhibited myelopoietic function of the bone marrow. Erythrocytes with micronuclei were markedly increased. Of the treated groups, the incidence of PCE in the group treated with 200 mg MDI/kg, decreased to 67% of that in the DMSO/corn oil control group. This statistically significant difference suggested inhibited myelopathic function of the bone marrow. There was no significant difference at the other concentrations. There was little difference between the DMSO/corn oil control group and the

treated groups with respect to erythrocytes containing micronuclei. It was concluded that MDI did not cause micronuclei, and therefore did not induce *in vivo* chromosomal aberration.

Another bone marrow micronucleus study has been reported in abstract form by Siegel et al. (1999), and reported in a literature publication by Zhong and Siegel (2000). Brown Norway rats (males, n = 6) were exposed, whole body, 2 at a time, to either 7 or 113 mg/m³ monomeric MDI condensation aerosol (MMAD 0.8 µm) for 1 hour, once a week for 3 weeks with sacrifice 1 week later. Micronuclei (MN) formation was assessed from bone marrow polychromatic erythrocytes (PCE). A dose-dependent increase in the frequency of micronucleated polychromatic erythrocytes (MN-PCEs) was noted: 1.5- and 4.5- fold increases of micronuclei were reported for the two exposure groups over control (a control group of 4 rats). No difference was found in the ratio of PCEs and NCEs between exposure and control groups, suggesting that there was no observable bone marrow cytotoxicity. The lack of interference from mast cell granules was confirmed using Acridine Orange staining of bone marrow smears from control and Brown Norway rats exposed to MDI or vincristine. However, the exposure to vincristine was not part of the study reported but is assumed to have been conducted in a separate and unreported study. Other assessments *in vitro*, in the same study, showed a significantly increased frequency of micronuclei in cultured hamster fibroblasts in response to MDA and thiol conjugates of MDI added to the incubation medium, but not to MDI. However, the *in vivo* protocol used was not standard for a micronucleus test, the findings are not consistent with the existing genotoxicity profile of MDI (or MDA), and target organ toxicity apart from the respiratory tract has not heretofore been seen in the many inhalation studies with MDI. It has to be reported that for this study, all MDI aerosols were generated by passing filtered dry air through MDI that was heated to 125°C. It should also be noted that in respective negative controls no heat source was used. This report from Siegel et al. (1999), Zhong and Siegel (2000) can not be fully evaluated without full details of the methodologies employed and the results obtained (e.g. no results were made available as to a positive control).

To fulfil the need for an acceptable *in vivo* micronucleus study on MDI, and to repeat to GLP and good scientific standards the procedures and observations of Siegel et al. (1999), a rat micronucleus study was designed, under the auspices of the International Isocyanate Institute (reported by Pauluhn and Gollapudi, 2001; published by Pauluhn et al., 2001). Four groups (6 per serial sacrifice 1, 2, 7 days after last treatment/exposure) of young adult male Brown-Norway rats (strain: BN/RijHsd) were either exposed whole-body (WB) to conditioned air (negative control) or to respirable aerosols of monomeric MDI at actual breathing zone concentrations of 9.2 ± 1.5 and 118 ± 8.6 mg/m³. One additional group was exposed to 110±14.4 mg/m³ MDI using a directed-flow nose-only (NO) mode. Animals were exposed 1 hour/day, one exposure/week for 3 consecutive weeks. For the WB exposed rats the principles of aerosol generation used by Siegel et al. (1999) were duplicated (condensation aerosol from heated MDI 125°C) whilst for the NO exposed group a combined dispersion-condensation aerosol principle was devised (dispersion aerosol from heated MDI 80°C), and evaporation/recondensation under controlled conditions). The MMAD for the WB exposed rats was in the range of 2.4 – 3.1 µm (GSD 1.6) whereas for the NO exposed rats the MMAD was 1.2 µm (GSD 1.5). Humidity ranged from ± 40% (WB) to ± 5% (NO). Neither the principle of aerosol generation nor the different humidity appeared to have any impact on the chemical stability of airborne MDI. Bone marrow smears were prepared 1, 2, and 7 days after the last exposure. Cells were stained with Acridine Orange and Wright Giemsa and MN-PCEs were counted. The ratio of polychromatic to normochromatic erythrocytes and the incidence of mast cells were also evaluated. Rats treated with cyclophosphamide (20 mg/ kg bw, gavage) and colcemid (4 mg/kg bw, i.p.) served as positive controls for clastogenic effects and for spindle poison effects, respectively.

All rats exposed to 0 (air) and 9.2 mg MDI/m³ tolerated the WB exposure without specific signs whilst the 118 and 110 mg MDI/m³ exposed rats showed signs of respiratory tract irritation and increased lung weights. Mortality did not occur in any group. Despite similar exposure concentrations, changes were most pronounced in NO-exposed rats.

No statistically significant increase in MN-PCEs was observed in either the whole-body or nose-only MDI-exposed groups at any sacrifice. Rats treated with the positive control substances showed statistically significantly increased MN-PCEs when compared to both the negative control and MDI-exposure groups. At no time point was there evidence of any conclusive effect on the frequency of mast cells. The low frequency of mast cells did not interfere with the identification and counting of MN-PCEs. Overall, with respect to the increase in MN-PCEs there were essentially no differences following staining with Acridine Orange or Wright Giemsa. Furthermore, no significant depression of PCEs among red blood cells (RBCs) was observed in the MDI-exposed groups whilst the positive control substances induced a significant depression in PCEs at the 48-hour sacrifice. At this time interval in these groups the PCE:NCE ratio was significantly decreased. An increase in PCEs, including the PCE:NCE ratio, of unclear relevance was identified in the nose-only MDI-exposure group when stained with Acridine Orange (24-hour sacrifice only).

These results indicate that aerosolised, inhaled MDI at concentrations as high as 118 mg/m³ air (a concentration high enough to produce portal-of-entry-specific toxic effects, including statistically significantly increased lung weights especially in NO exposed rats) did not induce cytogenetic damage *in vivo*.

It has to be mentioned that there is a concern that the bone marrow was not adequately exposed in this study in view of the lack of effects on the ratio of polychromatic to normochromatic erythrocytes (PN ratio) or other indications of systemic toxicity. However, referring to the Gledhill (2001a) study investigating the "Excretion and tissue distribution in the rat following inhalation exposure to [^{14}C]-MDI at 2 mg/m³ for 6 hours", it can be assumed there was indeed exposure of the bone marrow.

Other in vivo tests

Vock et al. (1995b) investigated the adduct formation of 4,4'-MDI or 4,4'-MDA with DNA or chromatin protein in dermally-exposed rats. Female Wistar rats were treated topically with [^{14}C] MDI on the clipped back. Adduct formation was investigated after [^{14}C] MDI and [^{14}C] MDA. After 24 or 48 hours, liver DNA and chromatin protein were isolated and their radioactivity determined. All samples were purified repetitively until constant specific activity was established. HPLC analysis of nucleotides from DNA of a MDA-treated animal resulted in quantitative recovery of the radioactivity in an adduct fraction, showing that no radioactivity was incorporated into nucleotides by DNA biosynthesis. The amount of radioactivity covalently bound to liver DNA was expressed in the units of the Covalent Binding Index, CBI = (µmol adduct/mol DNA nucleotide) per (mmol chemical administered/kg body weight) in order to estimate the DNA-binding potency. A CBI value of 1.7 was found for MDA after 24 hours. With MDI, the specific DNA radioactivity was too low to allow nucleotide analysis. Under the assumption that all radioactivity reflects adduct formation, after 48 hours a CBI value of 0.05 was calculated. Therefore, the maximum possible fraction becoming systemically available as MDA after topical treatment of rats with MDI was about 3%. The CBI values classify both MDI and MDA as very weakly genotoxic chemicals. Under allowable exposure conditions at the workplace, therefore, systemic MDI genotoxicity after skin contact is not considered a major toxic hazard as compared with sensitisation potency.

In another study done by Vock et al. (1995a), DNA adducts formed *in vitro* and in rat skin by MDI was analysed with the ^{32}P-Postlabelling method. Incubation of the 3'-phosphates of the deoxyribosides of cytosine (C), adenine (A), guanine (G) and thymine (T) with MDI in Tris buffer resulted in the formation of 5, 7, 8 and 2 reaction products, respectively. Incubation of DNA with MDI resulted in detectable levels of 5, 2 and 1 adducts attributable to C, A and G. Analysis of DNA isolated from the epidermis of rats treated dermally with 9 mg MDI showed an adduct pattern similar to the one seen in the *in vitro* DNA incubation. A total adduct level of 7 per 10^8 nucleotides was measured, the limit of detection was 2 adducts per 10^{10} nucleotides. The data indicate that a minute fraction of MDI can reach DNA *in vivo* in a chemically reactive form. In comparison with the genotoxic skin carcinogen 7,12-dimethylbez[*a*]anthacene on the other hand, the DNA-binding potency of MDI was more than 1,000-fold lower.

In a later study, tissues obtained from female Wistar rats exposed to a 0.9µm aerosol of 4,4'-MDI for 17 hours/day, 5 days per week, for one year, at levels of 0, 0.3, 0.7 and 2.0 mg/m^3, were analysed for DNA adducts (Vock et al., 1996). A ^{32}P-Postlabelling method was used to detect adducts formed by the reaction of the isocyanate groups of MDI with DNA and MDA with DNA. In the lung, neither isocyanate adducts nor the arylamine adduct were detectable. The same negative result was seen in the liver, the bladder, the kidney, the respiratory epithelium and the peripheral lymphocytes. In the olfactory epithelium, on the other hand, the arylamine-derived DNA adduct was detected, at the very low levels of 5, 9 and 10 adduct-nucleotides per 10^{10} nucleotides, for the three dose groups, respectively. The adduct cochromatographed with the one formed in the liver of rats after oral gavage of MDA. The results were discussed in terms of the importance of genotoxic versus non-genotoxic aspects of carcinogenesis.

The dose-response curves for the MDA-DNA adduct in the olfactory epithelium was supralinear. Interestingly, in the same animals, haemoglobin adduct levels showed the same shape of the dose-response curve. The ratio of protein adduct formation in erythrocytes and DNA adduct formation in a target tissue for toxicity, therefore, appeared to be constant over the dose range tested.

In the lung, the target organ for tumour formation in the rat, after inhalation exposure to MDI, no DNA-adduct was detectable in this study. Since MDI is able to react with MDA to form mixed ureas, the addition reaction occurring at the outer surface of the aerosol should result in the formation of chemically inert particles. So it is conceivable that factors such as chronic inflammatory response, cell injury and cell proliferation influenced the formation of tumours by MDI.

In a recent study by Vock and Lutz (1997), female Wistar rats were treated with [^{14}C] 4,4'-MDI in dried acetone on the back. Faecal excretion of radioactivity amounted to 20% of the administered radioactivity within 24 hours. Urinary excretion was below 1%. About 10% of the radioactivity was retained at the site of application. Epidermal nuclear protein exhibited very high specific radioactivity. ^{32}P-postlabelling analysis did not reveal isocyanate-DNA adducts. The nuclear protein radioactivity in the liver, lung and kidney was much lower than in the epidermis. DNA radioactivity in the liver was at the limit of detection. Conversion to the units of the Covalent Binding Index, CBI = (µmol adduct/mol DNA nucleotide) per (mmol chemical administered/kg body weight) resulted in a value of <0.1. The presence of 2% MDA in the application solution could have contributed about 0.03 CBI-units to the measured values. In comparison with genotoxic carcinogens, the upper bound value is indicative of a very weak maximum possible systemic genotoxic potency of topically administered MDI.

Human exposure

Holmén et al. (1988) studied 22 men working in the production and processing of flexible foam and 10 resin workers exposed to diisocyanates by cytogenetic methods (chromosomal aberrations, sister chromatid exchanges and micronuclei in lymphocytes) and by urinary mutagenic assays (thioester concentrations and mutagenic activity with *Salmonella typhimurium* TA98 and *Escherichia coli* WP2 uvrA). The referents were 20 men without any known occupational genotoxic exposure (most of them were lower white collars working in a mechanical factory). The workers from the flexible foam factory were exposed to TDI (personal air sampling showed <1-170 μg/m³ TDI in different tasks) but also amines (triethylenediamine, dimethylethanolamine, and N-methylmorpholine), polyols and chlorofluorocarbons were used. The resin production workers were exposed to TDI (4-52 μg/m³ in different tasks) and MDI (no measurements taken) used for modifying polyvinylchloride expanded by chlorofluorocarbons, with occasional use of tricresylphosphate and phthalic anhydride. The flexible foam workers (TDI and amine exposure) showed a significant increase (P=0.016) in thioester level in urine specimens collected in the afternoon of a working day, but chromosomal aberrations, SCEs, micronuclei in lymphocytes, and urine mutagenicity were increased, but not significantly, in workers of both groups, as compared with the referents.

The weight of this study is quite low due to the presence of several confoundings: e.g. 1) For the micronuclei, data are difficult to interpret as no current standard technique is used; 2) For SCE's, only 20 metaphases were scored, which is too low to detect a biologically relevant increase. Moreover, the control data are extremely high as compared with literature and no standard deviations are given; 3) In the results of chromosomal aberrations, a lot of gaps are included; 4) In the Ames test with urine samples, there was a high background revertant frequency for TA98. Criteria for a positive response were not met. Moreover, the authors themselves considered this study non-conclusive with regard to the question of a genetic effect of occupational exposure as measured by these cytogenetic parameters.

A case of a male worker engaged in rock consolidation by polyurethane in a coal mine, and exposed to MDI and its oligomers for about 5 years is described in Marczynski et al. (1992). He repeatedly suffered from asthmatic attacks. Before challenge testing he had not been occupationally exposed to MDI for 5 days. He was challenged with MDI containing 60% 4,4'-MDI, 30% various triisocyanates, and undefined isocyanates. In a challenge test he was continuously exposed to MDI at 3 different concentrations: 0.05 mg/m^3 for 15 min., 0.1 mg/m^3 for 30 minutes and 0.2 mg/m^3 for 15 minutes. The challenge test resulted in moderate immediate asthmatic reaction associated with significant hypoxaemia.

Polyacrylamide gel electrophoresis indicated double-strand breaks in white blood cell DNA after challenge with MDI. Some of the genomic DNA fragments were in the range of 100 bp. The additional bands found after denaturation, and rapid renaturation, could be taken to indicate cross-links in the smaller DNA fragments. The electrophoresis findings were supported by anion-exchange chromatography of white blood cell DNA. An additional peak appeared after challenge testing with MDI. As the worker had not been occupationally exposed to MDI for 5 days prior to the challenge tests, the results were taken to suggest transient changes in DNA. There was again indication of cross-linking as with electrophoresis. It was said that these effects of MDI could be due to its metabolites. The melting behaviour of the genomic DNA suggested alterations in the DNA after challenge with MDI, and leading to the observed decrease in hyperchromicity.

An altered apoptosis induced by MDI could not be excluded.

This study (Marczynski et al., 1992) has been strongly criticised on the grounds of the lack of checks for isolation artefacts, the inappropriateness of the test methods, the lack of suitable controls or standards, misinterpretation of the results, and the speculative nature of the conclusions (Gahlmann, 1993).

In a later study, (Marczynski et al., 1994a), blood samples were obtained from 10 isocyanate exposed workers both before, and after, provocation challenge with isocyanates. The white blood cells in the supernatant, and those in the pellet, were analysed by pulse field gel electrophoresis using a gentle agarose-plug method for DNA double-strand breaks. The chromosomal DNA of white cells in the pellets from blood samples of control patients, and which had been generated by apoptosis, was also analysed. The supernatant cells from blood cells before, or after, provocation did not give any indication of significant DNA double-strand breaks. In 6 of the 10 cases the cells in the pellets from blood samples after provocation contained more DNA fragments than those isolated before provocation. These fragments occurred in the region of 200-2,200 kilobases, and were not present in the DNA similarly isolated from the blood of control patients. It was concluded that the generation of the additional DNA fragments was related to cell death due to apoptosis, and that isocyanates exposure influenced the process of apoptosis.

In another study by Marczynski et al. (1994b), blood samples were frozen from 14 exposed workers who underwent workplace-related inhalative exposure tests of isocyanates of 0.05 to 0.1 mg/m^3 for one to two hours. Two fractions of isolated white blood cells (WBC) from supernatant (intact cells) and from the pellet (cellular debris) were separately investigated with the 'agarose plug' method by pulsed-field gel electrophoresis before and after diisocyanate exposure. In 8 out of 14 subjects, the *in vivo* results showed diisocyanate exposure to induce, in WBC from the pellet, additional degradation of chromosomal DNA into large fragments indicating an acceleration of apoptosis. DNA fragments were estimated to be in the region between the start (larger than 2,200 kbp) and 250 kbp. Using the laser densitometric method, about 30 to 40% more DNA fragments were found in damaged WBC 0.5 or 2.5 hours after diisocyanate exposure. 24 hours after diisocyanate exposure, most additional DNA fragments were removed. The authors concluded that occupational diisocyanate exposure can be associated with apoptosis.

The study by Vock et al. (1998) on double strand breaks in A549 cells provided evidence that the effects reported here by Marczynski are probably not related to genotoxicity but to cytotoxicity by necrosis. These observations regarding DNA fragmentation need to be confirmed by other studies in humans and more experimental work is required to understand their mechanism and significance.

In this context, it may be noted that in a workshop, Norppa (1999) reported results on the genotoxic effects of diisocyanate exposure among Finnish polyurethane foam workers. Cytogenetic biomarkers in peripheral lymphocytes were examined in 56 rigid polyurethane foam workers exposed to MDI, 17 flexible polyurethane foam workers exposed to TDI and 70 unexposed age- and sex-matched referents. MDI exposure resulted in a slightly increased frequency of sister chromatid exchanges. Bolognesi (1999) presented, during the same workshop, findings on the analysis of micronuclei in peripheral blood lymphocytes and buccal mucosa cells of the same Finnish polyurethane foam workers. A total of 41 MDI- and 17 TDI-exposed subjects and 56 referents were analysed for micronuclei frequency in lymphocytes; buccal mucosa samples were examined from 54, 17 and 64 subjects, respectively. In the MDI-exposed subjects, an increased micronuclei rate was evident in buccal cells of non-smoking subjects. The results from Norppa (1999) and Bolognesi (1999) were combined in an abstract presented by Norppa et al. (2000). The results presented by Norppa and Bolognesi

suggest that occupational exposure to MDI increases cytogenetic damage, indicating a possible genotoxic hazard of diisocyanate exposure. However, full publication of the findings has not yet occurred and the additional information provided was too poor for interpretation (absence of complete and comprehensive raw data). Additional information is still needed for a full understanding of the impact of the results in terms of hazard and/or risk assessment.

4.1.2.7.3 Summary

Overall, tests assessing the mutagenic potential of MDI *in vitro* and *in vivo* provide no convincing evidence of mutagenic and genotoxic activity. However, there are still some problematic issues in this area. First of all, many studies have not been conducted according to the highest methodological standards and the nature and purity of the test agents are not always clear.

One aspect that appears to have been resolved, relates to the positive Ames tests when using DMSO as a solvent for MDI. In these circumstances MDI was shown to be rapidly converted to the known mutagen MDA thus giving falsely positive results. (According to a report from industry, the same situation appears to hold when acetone was used as a solvent, but the data substantiating this are not available.) When an appropriate vehicle (EGDE) was used, MDI was shown not to be mutagenic by itself or in the presence of a biotransformation system. Eventually, the validity of excluding the contribution of MDA will depend on the outcome of the studies of the metabolic fate of MDI and its actual degree of conversion (or not) to MDA *in vivo* (see also summary, Section 4.1.2.1.3.).

One other problem relates to the *in vivo* micronucleus tests that have been performed. In one test (JETOC, 1982), the response in MDI-treated animals did not differ significantly from that in the control animals (given DMSO/corn oil), but the latter exhibited a high level of erythrocytes with micronuclei, thus possibly casting some doubt on the validity of the approach used for the test. In another study (Siegel et al., 1999; published as Zhong and Siegel, 2000), methodological aspects (including the fact that the micronucleus test was not designed according to standard protocols) preclude the interpretation of the possibly positive results. To fulfil the need for an acceptable *in vivo* micronucleus study on MDI, and to repeat to GLP and good scientific standards the procedures and observations of Siegel et al. (1999), a rat micronucleus study was designed, under the auspices of the International Isocyanate Institute and reported by Pauluhn and Gollapudi (2001). The results of this study indicate that aerosolised, inhaled MDI at concentrations as high as 118 mg/m³ air (a concentration high enough to produce portal-of-entry-specific toxic effects, including statistically significantly increased lung weights especially in NO exposed rats) did not induce cytogenetic damage *in vivo*.

Other studies that have investigated relevant endpoints, such as DNA-adduct formation, have not demonstrated any significant binding after topical or inhalatory exposure to MDI in animals.

Some human exposure studies have reported possible alterations in DNA status, but these data were obtained with uncertain methodologies and the results are not easy to interpret.

Table 4.33 Summary overview of studies on mutagenicity

Substance	Species	Method	Endpoint	Reference	Reliability[1]
Monomeric 4,4'-MDI	TA100 TA98	Ames-test, solvent: DMSO	-S9mix: neg. +S9mix: weak mutagen	Herbold, 1980a	3
Monomeric 4,4'-MDI	TA100	Ames-test, solvent: EGDE	-S9mix: neg. +S9mix: neg.	Herbold, 1990a	3
Monomeric 4,4'-MDI	TA100	Ames-test, solvent: acetone	+S9mix: mutagenic ED:20 µg/plate	Herbold, 1980c	3
Monomeric 4,4'-MDI	TA100 TA98	Ames-test, solvent: DMSO	+S9mix: ED: 100 µg/plate	Woolrich, 1982	4
Monomeric 4,4'-MDI	TA100 TA98	Ames-test, solvent: DMSO	-S9mix: neg. +S9mix: mutagenic TA100&TA98	Shimizu et al., 1985	3
Monomeric 4,4'-MDI	TA100 TA98 TA1535 TA1537	Ames-test, solvent: DMSO	-S9mix: neg. +S9mix: neg.	Zeiger et al., 1987	3
Monomeric 4,4'-MDI	TA100	Ames-test, solvent: DMSO	+S9mix: ED: 100 µg/plate mutagenic	Andersen et al., 1980	2
Polymeric MDI	TA100 TA98	Ames-test, solvent: DMSO	TA100-S9: neg TA100+S9:pos TA98-S9: neg TA98+S9: neg	Herbold, 1980b	3
Polymeric MDI	TA100	Ames-test, solvent: EDGE	TA100-S9: neg TA100+S9:neg	Herbold, 1990b	3
2,4'-MDI	TA1535 TA1537 TA100 TA98	Ames-test, solvent: DMSO	-S9mix: neg. +S9mix: neg.	Herbold, 1996a	2
2,4'-MDI	TA1538 TA100 TA98	Ames-test, solvent: DMSO	TA98+S9: ED: 15 µg/plate TA100+S9: ED: 30µg/plate weak mutagenic	Herbold, 1996c	3

Table 4.33 continued overleaf

Table 4.33 continued Summary overview of studies on mutagenicity

Substance	Species	Method	Endpoint	Reference	Reliability[1]
Generic MDI	TA1535 TA1537 TA100 TA98	Ames-test, solvent: DMSO	-S9mix: neg. TA100+S9: pos. TA98+S9: pos.	Herbold, 1996b	3
4,4'-MDI; 2,4'-MDI; Polymeric MDI; Mixture of isomers of monomeric MDI	TA1535 TA1537 TA1538T A100 TA98	Ames-test, Solvents: DMSO EDGE	-EDGE ± S9: negative -DMSO – S9: negative -DMSO + S9 in TA 1535, 1537: negative -DMSO + S9 in TA 98 , 100: positive (2,4'-MDI positive in TA1538)	Herbold et al., 1998	2
Monomeric 4,4'-MDI; Polymeric MDI; Generic MDI; 2,4'-MDI	TA1535 TA1537 TA100 TA98	Ames-test, Solvents: DMSO EDGE and Stability studies	-EDGE ± S9: negative -DMSO – S9: negative -DMSO + S9 in TA 1535, 1537: negative -DMSO + S9 in TA 98 , 100: positive	Seel et al., 1999	2
Monomeric 4,4'-MDI	Mouse L5178Y	Specific locus mutation assay; modified Clive et al (1972/1977) solvent: DMSO	≥1,000µg/ml: cytotoxic ≥100µg/ml: precipitation +S9: ≥200µg is mutagenic (90%tox)	McGregor et al., 1981a	3
Polymeric MDI	Mouse L5178Y	Specific locus mutation assay; modified Clive et al (1972/1977) solvent: DMSO	≥106 µg/ml: cytotoxic; not mutagenic	McGregor et al., 1981b	3
Technical grade MDI	Human whole blood lymphocytes	Sister Chromatid Exchange (SCE)	chromos. aberrations:pos +S9: 4.30 µg/ml -S9: 0.54-4.30 µg/ml SCE: marginally ±S9: 4.30 µg/ml	Mäki-Paakkanen et al., 1987	3

Table 4.33 continued overleaf

Table 4.33 continued Summary overview of studies on mutagenicity

Substance	Species	Method	Endpoint	Reference	Reliability[1]
Monomeric 4,4'-MDI	BHK21C13 cells hamster	Transformationtest see, Styles, 1977 solvent: DMSO	±S9: increased transformation frequency	Poole et al., 1980a	3
Polymeric MDI	BHK21C13 cells hamster	Transformationtest see, Styles, 1977 solvent: DMSO	±S9: increased transformation frequency	Poole et al., 1980b	3
BisGS-MDI, BisCYS-MDI MDI	V79 cells hamster	MN assay using immunofluorescent staining of kinetochore in MN of cytokinesis blocked V79 cells	MN formation mediated through disruption and/or by affecting the function of the mitotic spindle. MDI: negative	Zhong et al., 2001	3
Monomeric 4,4'-MDI	Mouse	modified Micronucleus test (Heddle & Schmid, 1971/1973) solvent: DMSO/corn oil	no chromosomal aberrations *in vivo*	JETOC, 1982	3
Monomeric 4,4'-MDI	Brown Norway rats	Bone marrow micronucleus study	Increase of micronuclei	Siegel et al., 1999 Zhong and Siegel, 2000	4 3
Monomeric 4,4'-MDI	Brown Norway rats	Bone marrow micronucleus study	No cytogenetic damage *in vivo*	Pauluhn and Gollapudi, 2001; Pauluhn et al., 2001	2
MDI No more data	Human	Human blood: - micronuclei - SCE's - chromosome aberrations Human urine: - Ames	Non conclusive with regard to the question of a genetic effect of occupational exposure	Holmén et al., 1988	3
MDI no more data	Human	case reports: DNA adducts	Apoptosis and genotoxicity	Marczynski et al., 1992, 1994a, 1994b Gahlmann, 1993	3

Table 4.33 continued overleaf

Table 4.33 continued Summary overview of studies on mutagenicity

Substance	Species	Method	Endpoint	Reference	Reliability[1]
Monomeric 4,4'-MDI	Rat	^{32}P-Postlabelling analysis of DNA adducts	Adduct formation in dermally-exposed rats : very weakly genotoxic; After inhalation: only MDA adducts in olfactory epithelium	Vock et al., 1995a, 1995b, 1996	3 2 2
Monomeric 4,4'-MDI	Rat	DNA adduct formation of [14C]-4,4'-MDI after topical application	Very weak maximum possible systemic genotoxic potency	Vock and Lutz, 1997	2
Monomeric 4,4'-MDI	A549 cells human	Induction of DNA double-strand breaks	No genotoxicity but cytotoxicity by necrosis	Vock et al., 1998	3
MDI no more data	Human	SCE micronuclei	Slightly increased frequency of SCE ; increased micronuclei rate in buccal cells	Norppa, 1999 Bolognesi, 1999 Norppa et al., 2000	4 4 4

1) Reliability key: 1 = method and description are in accordance with test guidelines
2 = falling short of highest standards concerning aspects of protocol or reporting
3 = method or report not in accordance with test guidelines
4 = minimal description of method and report

4.1.2.8 Carcinogenicity

4.1.2.8.1 Studies in animals

Oral

No carcinogenicity studies were available using the oral route of exposure.

Dermal

No carcinogenicity studies were available using the dermal route of exposure.

Inhalation

A 2 year toxicity/carcinogenicity inhalation study with polymeric MDI aerosol was carried out in 4 groups of 70 male and 70 female Wistar (Cpb:WU) rats each (Reuzel et al., 1990, 1994a). Each group was subdivided into a satellite group of 10 rats/sex and a main group of 60 rats/sex. The rats were exposed (whole body) to target concentrations of 0, 0.2, 1.0 and 6.0 mg polymeric aerosol/m^3 for 6 hours/day, 5days/week during a period of one year for the satellite groups and 2 years for the main groups. The method used was in accordance with OECD Guideline n° 453 and 87/302/EEC.

Animals were observed daily for clinical signs and for changes in behaviour and mortality just before and after exposure and once a day on non-exposure days. Rats were weighed weekly for the first 13 weeks and every 4 weeks thereafter. Rats were also weighed at day of autopsy. Haematological parameters and urinary parameters were measured in all rats of the satellite group in week 52. Biochemical blood parameters were measured in blood samples taken at euthanisation from all rats of the satellite groups. Animals of the main groups were euthanised in weeks 105 and 106, autopsied, and examined for gross pathological changes. Adrenals, brain, heart, kidneys, liver, lungs, with mediastinal lymph nodes, trachea and larynx, spleen, and testes of all rats of the satellite groups and all survivors of the main groups were weighed. In the 1-year (satellite) study groups histopathological examination was carried out of kidneys, liver, nose, larynx, trachea with bronchi, lungs, mediastinal lymph nodes, and all gross lesions of the rats. In the 2-year (main) study groups 43 different organs or tissues and all grossly visible lesions were examined by light microscopy of the control and high-concentration animals and of the low and mid-concentration decedents. Moreover, in the low and mid-concentration survivors of the main groups, nose, lungs, mediastinal lymph nodes, and all gross lesions were subjected to histopathological examination.

The composition and impurities found in the polymeric MDI test material were determined by Bayer (the supplier). The concentration data on trace impurities are: hydrolysable chlorides, < 0.3%w/w; chlorobenzenes, < 0.015% w/w; phenyl isocyanate, 0.004 ± 0.001% w/w; 2,4'-MDI, 2 – 3% w/w. The actual mean concentrations of polymeric MDI aerosol in the different test atmospheres were: 0, 0.19, 0.98 and 6.03 mg/m^3. 95% of the particles were <5µm.

The effect of chronic exposure of rats to respirable polymeric MDI aerosol was confined to the respiratory tract. Compound-related changes were found in the nasal cavity, the lungs and the mediastinal lymph nodes, and to some degree they were already present after 1 year of exposure. These changes were characterised by increased lung weights, grossly observed spotted lungs, and histopathogical alterations in the nasal cavity, the lungs and the mediastinal lymph nodes.

Mortality incidences in males were comparable in all groups. Female rats showed negatively concentration-related mortality incidence. The number of animals with palpable masses did not differ between test and control animals. No treatment-related differences in body weights were observed between control and test groups. Haematological examination of rats at day 357-358 revealed no exposure-related differences between groups. Biochemical examination (clinical chemistry) performed on days 360, 366 and/or 367 was essentially negative. No alterations were observed from parameters measured in urine of rats exposed to polymeric MDI aerosol for 1 year. Lung weights were statistically significantly increased in both males and females exposed to 6.0 mg/m^3 for 1 or 2 years. No treatment-related gross changes were found in animals exposed for 1 year. Gross examination of animals exposed for 2 years revealed increased incidence of lungs with spotted surface and/or discoloured appearance in male rats exposed to 6 mg/m^3.

At microscopy the following changes were observed:

- increased incidence of rats with a higher degree of basal cell hyperplasia frequently accompanied by hyperplasia of Bowman's glands in the olfactory epithelium in the nose at levels of 1 and 6 mg/m^3.
- accumulation of alveolar macrophages containing MDI-associated material at the level of the alveolar duct in the lungs at all exposure levels.
- an increased incidence of calcareous particles in the lungs at the level of 6 mg/m^3.

- alveolar duct epithelialisation (replacement or transformation of flat epithelium into cuboidal epithelium) as well as fibrosis in tissues surrounding the macrophage accumulations at levels of 1 and 6 mg/m^3.
- an increased incidence of localised areas of alveolar bronchiolisation: bronchiolar/alveolar hyperplasia at a level of 6 mg/m^3; bronchiolar at a level of 1 mg/m³.
- 8 pulmonary adenomas (6 males and 2 females) and 1 pulmonary adenocarcinoma (1 male) from the 6 mg/m^3 exposure group; no lung tumours were observed in the 1.0 or 0.2 mg/m^3 exposure groups. The incidence and distribution of other tumour types was not affected by treatment.

From the results of the study it was concluded that the no-adverse-effect level for the toxicity of polymeric MDI was 0.2 mg/m^3. In addition, exposure to a level of 6 mg/m^3 polymeric MDI was related to the occurrence of pulmonary tumours.

In another long-term inhalation study over a maximum of 24 months including satellite groups with 3, 12, and 20 months exposure, by Hoymann et al. (1995), the chronic toxicity and the carcinogenicity of monomeric 4,4'-MDI (Bayer, purity: a minimum of 99.5%) were investigated. Female Wistar rats (Crl:[Wi]Br), in groups of 80 animals, were exposed in 6 m^3 inhalation chambers for 17 hours/day, 5 days/week to 0.23, 0.70, and 2.03 mg/m^3 MDI in aerosol form, a control group was exposed to 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, a moderately 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 (at 2.05 mg/m^3) were ascertained.

It has to be noted that there was an untypical high mortality due to tumours of the pituitary gland, in all groups (control group included), which limits the evaluation of this study. With this study it cannot be clarified whether prolonged exposure would have resulted in tumour formation (III). Another point for discussion is the exposure scenario (17 hours/day). A 17-hour daily exposure is unusual and prevents normal spontaneous healing processes after contact with irritating substances. As it is well known that the used concentrations are locally irritating (irritation was shown in all dose groups), this again limits the evaluation of the study. However, as noted in Section 4.1.2.6.1, the comparison of the Reuzel et al. (1990,1994a) and the Hoymann et al. (1995) studies under the auspices of the III and published as a review by Feron et al. (2001) has shown close agreement between the findings reported.

Feron et al. (2001) concluded that the major pulmonary effects included increased lung weights together with bronchiolo-alveolar adenomas and hyperplasia, and interstitial fibrosis which occurred consistently in both studies, indicating a very similar quantitative response of the lungs to polymeric and monomeric MDI. The quantitative response of the lung was clearly dose-related in each study, and when the studies were considered as a whole a reasonable overall dose-response relationship was apparent for major lung lesions. In both studies lung tumours were only seen in the top dose group. In the Reuzel et al. study, the only treatment-related effect seen at the lowest exposure level (0.2 mg/m³) was the occurrence of particle-laden alveolar macrophages. These macrophages had a normal appearance without any sign of degeneration. Therefore (according to Feron et al., 2001) this macrophage reaction is considered a physiological response to the deposition of particles in the lungs. Since, moreover, in the lowest dose group the presence of these viable particle-laden macrophages was not seen to be

accompanied by any tissue damage or inflammatory reaction, the lowest dose examined in the Reuzel et al. study (0.2 mg/m³) was regarded as a NOAEL.

4.1.2.8.2 Studies in humans

Cancer incidence and mortality patterns were investigated in a cohort of 4,154 workers employed in Swedish polyurethane foam manufacturing plants for at least 1 year (Hagmar et al., 1993b). Flexible foam blocks had been made in 3 plants, with initially storage of the blocks in the production premises for 24 hours, but more latterly storage in a separate room for 3 days. The remaining 6 plants had manufactured dead cast mouldings with further handling usually being located within the same premises. Workers other than moulders were therefore exposed to isocyanates.

Each workplace/job task was categorised for each calendar year, by an experienced occupational hygienist, and in relation to the categories 'no exposure', 'low or intermittent exposure', or 'apparent exposure' to MDI and TDI. The small size of the 'low or intermittent exposure' group in person-years led to its exclusion from the further analysis. TDI had been used in all the plants and MDI in all but one, so that it was impossible to evaluate their individual effects. Airborne exposure to the isocyanates had been measured on 6-18 occasions at each plant on 7-24 days. The time-weighted average levels of TDI had normally been < 100 mg/m^3 and were currently 20 $\mu g/m^3$. The corresponding values for MDI had been < 10 $\mu g/m^3$. However, much higher values had been repeatedly measured up to 3 mg/m^3 for TDI, and up to 0.35 mg/m^3 for MDI. There was also ill-defined exposure to blowing agents, mould lubricants, amine accelerators, and various organic solvents.

As a control expected mortality rates were calculated using calendar year, gender, and a five-year age-group, specific mortality rates for Sweden. Similarly, yearly incidences of cancers were obtained from the National Swedish Tumour Register. In either cases death, emigration, or the 80^{th} birthday were used as individual endpoints, whichever occurred first.

The observed, statistically significant deficit in all-cause mortality was reduced when excluding the first 10 years of exposure, and this was ascribed to the healthy worker effect. There was no increased risk of death caused by bronchial obstructive disease. The reduced incidence, against control, of all malignant neoplasms was almost statistically significant. Within this there were slight, statistically insignificant increases in the incidences of rectal cancer and non-Hodgkin's lymphoma, with a larger increase against control being observed when the first 10 years of exposure were excluded. The 'no exposure' group had fewer rectal cancers than the expected whereas the 'apparent exposure' group had more than expected. A similar smaller difference was seen with non-Hodgkin's lymphoma. When a minimum latency period of 10 years was applied the increases against control were even higher, but there were very few cases.

As the cohort was young with relatively short exposure to the isocyanates, future studies would allow more conclusive evaluation. A case referent study within the cohort was made to assess more thoroughly the association between exposure to TDI or MDI and risk of cancer (Hagmar et al., 1993a). The study was mainly performed to diminish the exposure misclassification that was obvious in the cohort study.

The main findings of this case referent study was that the tentative associations, derived from the previous cohort study, between exposure to isocyanates and excess risk for non-Hodgkin's lymphomas and rectal cancer were not supported. Instead, non-significant associations with prostate cancer and possibly colon cancer were seen.

A retrospective mortality and cancer morbidity study was conducted to investigate associations between health risk and exposures from polyurethane foam production, particularly exposures to diisocyanates including MDI (Sorahan and Pope, 1993). The study population was taken from 11 factories in England and Wales that had begun manufacturing foam before 1980. TDI was the principal isocyanate used; MDI represented about 5% of the amount of TDI. Prior to 1970 only TDI was used.

The cohort consisted of 8,288 workers, of whom 2,465 were women, employed for at least 6 months and having a portion of this employment in the period 1958-1979. Job histories and descriptions were obtained from personnel records. All jobs were classified into diisocyanate exposure levels: high, low, minimal/zero, and unclassifiable. Job history data for each worker were matched to job exposure classification to assign person-years of follow-up to three time-dependent exposure levels. The minimum length of follow-up was 9 years. Only 2% of the cohort was lost to follow-up.

Death certificates were obtained for 803 of the 823 known deaths and were coded according to the 9th revision of the International Classification of Diseases. The National Health Service Central register provided information on 277 incident cancers from 1971 through 1988, the year in which the study ended.

Cause-specific Standardised mortality ratios (SMRs), concentrating on cancer causes, were calculated by applying gender-specific external rates for England and Wales. Separate SMRs for certain cancers were also presented by gender, by years since first hire, and by relationship to cigarette smoking. The SMR for all cancers was 88 (95% CI=84-100), based on 221 observed and 251.4 expected deaths. For females, a statistically significant excess of pancreatic (SMR=271) and lung (SMR=176) cancer was calculated. However, no trend was seen with time since first hire for either of these cancers. When cancers related to smoking were grouped separately from those unrelated to smoking, the SMRs for women in the entry cohort were 263 for cancers related to smoking, and 67 for cancers unrelated to smoking. Available smoking information as of 1981 on a subset of the study population women revealed that 58% of them were smokers, contrasted with 37% of women in England and Wales in 1980. It is therefore conceivable that the high SMR for cancers related to smoking was due to a higher smoking prevalence in women of this cohort.

The relationship between diisocyanate exposure and mortality from all causes of death, respiratory diseases, lung cancer, and pancreatic cancer was further evaluated via a multiplicative relative risk model. The internal referent group was person-years at the reference level of all covariates. Exposure was not a statistically significant addition to the model for any of the 4 causes. There was some suggestion of increased risk at the higher exposure level for lung cancer with a relative risk of 126 (95% CI=39-409) and respiratory diseases (95% CI=54-573). None of the females who died had been classified as receiving either higher or lower diisocyanate exposure during any of their employment history. The authors concluded that excesses in female cancer were probably due to cigarette smoking and other factors unrelated to diisocyanate exposure. Although this well-designed study did not find an association between exposure to diisocyanates and cancer, the cohort was young and follow-up was relatively short.

Updated findings of the study reached the rapporteur as an III draft report (Sorahan and Nichols, 2001), and are recently published (Sorahan and Nichols, 2002). The mortality (1958-1998) and cancer morbidity (1971-1994) experienced by the same cohort of 8,288 male and female employees (all employed for at least 6 months with some period of employment in the period 1958-1979) from the 11 factories in England and Wales engaged in the manufacture of flexible polyurethane foams were investigated. Two analytical approaches were used, indirect

standardisation and Poisson regression. The update of the Sorahan cohort adds 10 years of follow-up. The cohort is a mixture of entry cohorts (n = 5,321) and survivor populations (n = 2,967); the latter comprising those workers in employment on the earliest dates for which complete personnel records were still available. With 20% of the cohort deceased, this is the most mature cohort. Compared with the general population of England and Wales, mortality from lung cancer in female employees was significantly elevated (observed 35, expected 19.4, SMR 181, P<0.01) but the increase is similar to that seen in the first study (SMR 181 versus 179 previously). A similar excess was not found for male employees (observed 134, expected 125.0, SMR 107). There were no significantly elevated cause-specific SMRs among the sub-cohort (n = 1782) with some period of isocyanate-exposed employment. No significant positive trends were found between risks of lung cancer or risks of non-malignant diseases of the respiratory system and durations of 'lower' or 'higher' exposures to diisocyanates. The study has been unable to link isocyanate-exposed employment either with risks of lung cancer or with risks of non-malignant diseases of the respiratory system. According to the authors, the almost doubled SMR for female lung cancer is most likely due to factors unrelated to the industry under study. However, these factors are not specified, and this finding requires further scrutiny. Unfortunately, it was not possible to evaluate the role of smoking habits or dietary factors in these findings.

It may be concluded from these studies that occupational exposure to isocyanates did not cause an overall increased risk of cancer in the Swedish or British polyurethane industry. Nevertheless, continued follow up would allow more definite conclusions to be drawn and more epidemiological surveillance should also be conducted to confirm the lack of carcinogenicity.

A single case report describes very briefly a case of lung cancer, which developed in the course of an occupational chronic bronchopulmonary disease due to isocyanates (Mortillaro et al., 1982). The isocyanate worker, a non-smoker, had been exposed to MDI and TDI for 15 years. No data are available to substantiate a causal relation between the lung cancer and the exposure to MDI. No other case reports have confirmed this anecdotal observation.

It has to be stated that these studies are considered to be limited by indications that exposure was not confined to MDI, but to isocyanates in general, with MDI exposure where measured, being a small fraction of the total exposure.

4.1.2.8.3 Possible mechanisms of carcinogenesis

The pathogenesis as proposed by Reuzel et al. (1994a), for the polymeric MDI-associated lung effects including tumours is as follows: The initial event is cytotoxicity to cells, especially Type I pneumocytes, lining the centriacinar region of the lung following contact with polymeric MDI aerosol. Alveolar macrophages phagocytise the foreign material. However, focal denudation of the epithelium leaves basement membranes exposed and/or damages the interstitium. Repair of both interstitium (by fibroblasts) and the epithelium ensue. Phagocytes of test material by activated macrophages may lead to elaboration of growth factors for both fibroblasts and epithelial cells, primarily Type II pneumocytes. Fibroblast and epithelial proliferation occur. Hyperplasia of Type II pneumocytes progresses, perhaps mediated by factors from alveolar macrophages. This progress continues until a small number of lung adenomas occur in the areas of most florid Type II pneumocyte hyperplasia and macrophage accumulation. The progression to adenoma and/or adenomacarcinoma may involve input from an increased background mutation rate secondary to the prolonged Type II cell proliferation. The above explanation was supported by the findings at the exposure level of 1 mg/m^3/day where there was minimal irritation and non-neoplastic changes in the lung were slight. No tumours were found. Thus

according to the authors, exposure to polymeric MDI at levels which do not result in recurrent tissue damage should not produce tumours.

Although oncogenesis on the basis of irritation, inflammation and increased cell proliferation could not be excluded in the study by Hoymann et al. (1995), the authors considered that tumours possibly resulted from the formation of MDA. The latter was suggested by the finding of haemoglobin adducts and exposure-related urine concentrations of 4,4'-MDA and from the fact that MDA is carcinogenic in animal studies. The MDI- and MDA-DNA-adduct findings, respectively, were negative in the lung, in contrast to the nose, but this could be due to the method used. The assumptions made by Sepai et al. (1995b) were considered in point in Section 4.1.2.1.1.

However the publication by Day et al. (1997) on TDI suggests that a different mechanism of haemoglobin or protein adduct formation could equally be anticipated. The first step might be the formation of a labile adduct between MDI and GSH. In a subsequent step the isocyanate is transferred to more stable adduct with larger proteins. This mechanism seems to be more straightforward than the rather complicated sequence of reactions via the nitroso compound. This GSH transfer mechanism does not require the assumption that free MDA must occur as intermediate metabolite.

Two short-term studies, one over a 2 week period (Pauluhn et al., 1999) and the other over a 4-week period which included also a one month recovery period (CTL report from 1999: published as Kilgour et al., 2002) have been conducted with the objectives of identifying parameters indicating cellular stress and to verify if the LOEL's for the short and long term studies differ markedly. The design of the studies can be summarised as follows:

Table 4.34 Study comparison between Pauluhn et al., 1999 and CTL, 1999

	Pauluhn et al.			CTL		
Test substance	Polymeric MDI			Polymeric MDI		
Exposure duration	6 hours/day; 5 days/week first week; Daily for a second week			6 hours/day; 5 days/week; 4 weeks		
Mode of exposure	Directed-flow nose only			Nose only		
Mean analytical concentration (mg/m³)	1.1	3.3	13.7			
Gravimetric concentration (mg/m³)	1.2	4.9	17	0.93	3.88	10.3
Cumulative concentration (mg h/m³)	86	353	1,224	134	559	1,483
Particle size MMAD (µm) GSD	1.46 1.51	1.47 1.49	1.52 1.60	0.88 2.47	1.20 1.95	1.09 1.68
Rat strain	Wistar : strain Hsd Cpb:WU (SPF)			Wistar : strain Alpk:APfSD		
Sex and numbers	17 males and 17 females			10 females		
Recovery groups	None			10 females		

The findings of these studies can be summarised as follows:

- Changes in lavage cell numbers and cytology were observed at both the intermediate and high exposure levels (after 4 weeks only).

- In both studies, many macrophages appeared 'foamy' at the two higher concentrations and analysis showed increased phospholipid content, indicative of interference with pulmonary surfactant.
- Measurement of lavage fluid parameters (total protein, alkaline phosphatase, β-N-acetylglucosaminidase, acid phosphatase, lactate dehydrogenase, γ-glutamyl-transpeptidase, total phosphatidylcholine) demonstrated a response in the high exposure level only.
- Cell proliferation in the terminal bronchiolo-alveolar regions was increased in a concentration related manner at all concentrations in both studies. Light microscopy complemented the findings in bronchiolar lavage fluid.
- Electron microscopy demonstrated intralysosomal inclusions in alveolar macrophages, Type II cell hyperplasia with increases in number and size of lamellar bodies and increased intra-alveolar surfactant and debris in the high exposure level. Some of these changes were seen also in the intermediate exposure group.
- Following the 30-day recovery period, all observed responses had returned to normal.

The results are consistent with pulmonary/cellular stress in response to chemically reactive particulates. These findings suggest that an exposure concentration of 1 mg/m³ (duration of exposure 6 hours/day) caused non-specific cell proliferation of Type II pneumocytes. This is consistent with the pathological changes in the 2 year bioassays and may be indicative of a non-genotoxic mechanism of tumour formation.

Pauluhn (2000) examined the acute pulmonary response of female Wistar rats (n = 6, except for the 20 mg MDI/m³ concentration n = 7) exposed nose-only to respirable polymeric MDI aerosol (nebulised). Based on the above described mechanistic hypothesis, this study investigated the time course of the relationship between acute pulmonary irritation and ensuing disturbances of the air/blood barrier in rats exposed to concentrations of 0 (conditioned dry air), 0.7, 2.4, 8, or 20-mg MDI/m³. The total duration of exposure was 6 hours. The time-response relationship of MDI-induced acute lung injury was examined at 0 hours (directly after cessation of exposure), 3 hours, 1 day, 3 days, and 7 days after exposure. Bronchoalveolar lavage (BAL) fluid was analysed for markers indicative of injury of the bronchoalveolar region (angiotensin-converting enzyme, protein, alkaline phosphatase, lactate dehydrogenase, γ-glutamyltranspeptidase, and sialic acid). Phosphatidylcholine and acid phosphatase were determined in BAL fluid and cells. Glutathione was determined in BAL fluid and lung tissue. This analysis revealed no latent period of effects except a delayed influx of cells and increased lung weights on postexposure days 1 and 3. Markedly loaded BAL cells with phosphatidylcholine were observed on day 1 only. In most instances, changes returned to the level of the air exposed controls on day 7, except increased glutathione in lung tissue. The findings suggested that the most sensitive markers of dysfunction of the air/blood barrier were angiotensin-converting enzyme, protein, and alkaline phosphatase. The statistically significant increase in intracellular phosphatidylcholine and decreased intracellular acid phosphatase on the exposure day suggested that increased amounts of phospholipids are phagocytised by alveolar macrophages, associated with protracted lysosomal catabolism. Partially glutathione-depleted rats exposed to 20 mg/m³ experienced a more pronounced increase in BAL protein than normal rats. Pauluhn (2000) suggested that respirable polymeric MDI aerosol interacts directly with the air/blood barrier causing increased extravasation of plasma constituents as a result of increased permeability of capillary endothelial cells. A transient dysfunction of the pulmonary epithelial barrier occurred at a level as low as 0.7 mg/m³ and appeared to be related to a dysfunction of pulmonary surfactant. Non-protein sulfhydryl constituents appeared to play a role as portal-of-entry specific modifying factors.

According to the author the findings of this study support the view of Reuzel et al. (1994a) that epigenetic (mitogenic) mechanisms should be primarily involved in neoplasia formation at this level of exposure.

Kilgour et al. (2002) reported (combined with the subacute inhalation study already discussed – CTL report, 1999) an acute inhalation study designed to evaluate early changes in the lungs of rats (Wistar) resulting from exposure to polymeric MDI aerosols, and to assess recovery from the observed effects of exposure. Groups of 40 female rats were exposed (nose-only) to target concentrations of 0, 10, 30, or 100 mg/m³ polymeric MDI for 6 hours. At 1, 3, 10, or 30 days following exposure, 5 rats from each group were taken for analysis of lung lavage components and 5 for pathological examination.

Acute exposures produced clinical signs in all animals that were consistent with exposure to irritant aerosols (abnormal respiratory noise, breathing rate reduced and depth increased, mucous secretions from the nose). An exposure concentration-related body weight loss and increase in lung weight were seen post-exposure, with complete recovery by day 10. Immediately following exposure there were increases in total cells, total protein, alkaline phosphatase, NAG and some indication of increased LDH activity in lung lavage fluid. By day 3 post-exposure, further increases were apparent in total cell counts. LDH activity was elevated in all groups to a greater extent than on day 1 post-exposure, although alkaline phosphatase and NAG activity had returned to control levels. Increases in cell replication became apparent in both the terminal bronchioles and centro-acinar alveolar regions examined, the response being concentration-dependent, correlating with the concentration-dependent bronchiolar hyperplasia seen histologically and type II cell hyperplasia identified by electron microscopy. By day 10 post-exposure, most of the measured parameters had returned to control levels. Cell proliferation was still slightly higher than control levels in the 30 mg/m³ group. At the light microscopy level, macrophage accumulations were still evident in animals exposed to 10 mg/m³ only, epithelialisation of the alveoli was present in animals exposed to 30 and 100 mg/m³ and thickening of the alveolar wall and ducts were evident in animals exposed to all concentrations, although generalised effects had resolved to a large extent. By day 30 post-exposure, lung weights, lung lavage parameters, cell proliferation and ultrastructural appearance had returned to normal at all exposure concentrations. Some slight epithelialisation of the alveolar duct and cell exudate in the lumen was still evident at low incidence in the 100 mg/m³ group, but all other effects had recovered. The time course of changes in the lung over the initial days following exposure consisted of a pattern of initial toxicity, rapid and heavy influx of inflammatory cells and soluble markers of inflammation and cell damage, increased lung surfactant, a subsequent recovery and epithelial proliferative phase and, finally, a return to the normal status quo of the lung. During these stages there was evidence of perturbation of lung surfactant homeostasis, demonstrated by increased amounts of crystalline surfactant and increased number and size of lamellar bodies within type II alveolar cells.

In summary, according to the authors, a single acute exposure of rats to respirable aerosols of polymeric MDI, resulted in a pattern of lung responses that is entirely consistent with exposure to irritant aerosols.

Further investigations are necessary to clarify the mechanism of the MDI-dependent tumour induction in the respiratory tract of the rat.

4.1.2.8.4 Summary

First it has to be mentioned that the only carcinogenicity data relate to the inhalation route of exposure.

In the 2-year animal inhalation study by Reuzel et al., (1990, 1994a) no adverse effect on the distribution and incidence of tumours apart from tumours in the lungs were found. According to the authors, and compatible with the hypothesis of Pauluhn et al. (1999), Pauluhn (2000) and Kilgour et al. (2002), the pulmonary tumours develop secondary to the irritation by polymeric MDI aerosol. Hyperplasia of Type II alveolar cells is a common non-specific reaction to many forms of toxic lung injury. It is commonly accepted (though not proven for the lung) that such processes can produce tumours through non-genotoxic (epigenetic) mechanisms.

In the long-term animal inhalation study by Hoymann et al. (1995), a single bronchio-alveolar adenoma was found at 2.05 mg/m^3 MDI. An indication of a possible mechanism of the oncogenesis resulted from the haemoglobin adducts and urine concentrations of 4,4'-MDA and from the fact that MDA is carcinogenic in animal studies. On the other hand, oncogenesis on the basis of irritation, inflammation and increased cell proliferation cannot be excluded.

Further investigations seem to be necessary to clarify the mechanism of the MDI-dependent tumour induction in the respiratory tract of the rat.

Feron et al. (2001) concluded in a review of the Reuzel et al. (1990, 1994a) and the Hoymann et al. (1995) studies that low incidences of lung tumours occurred at the highest dose level in both studies but no lung tumours were found at lower dose levels. For inflammatory and other non-neoplastic pulmonary changes, establishment of a NOAEL of 0.2 mg/m³ (exposure 6 hours/day, 5 days/week for 24 months) was considered scientifically justifiable for both polymeric and monomeric MDI.

There is inadequate evidence of carcinogenicity in humans and limited evidence in experimental animals.

Table 4.35 Summary overview of studies on carcinogenicity

Substance	Species	Method	Endpoint	Reference	Reliability[1]
Polymeric MDI	Rat	chronic inhalation and carcinogenicity test	pulmonary adenoma and adenocarcinoma LOAEL: 6 mg/m³	Reuzel et al., 1990, 1994a	1 2
Monomeric MDI	Rat	chronic inhalation and carcinogenicity test	a single bronchio-alveolar adenoma at 2.05 mg/m^3	Hoymann et al., 1995	2
MDI no more data	Human 1 case		lung cancer	Mortillaro et al., 1982	4
MDI no more data	Human survey		no increased risk of lung cancer	Hagmar, 1992, 1993a, 1993b,	3

Table 4.35 continued overleaf

Table 4.35 continued Summary overview of studies on carcinogenicity

Substance	Species	Method	Endpoint	Reference	Reliability[1]
MDI/TDI	Human survey		no increased risk of lung cancer	Sorahan and Pope, 1993 Sorahan and Nichols, 2001, 2002	3 3
Monomeric MDI Polymeric MDI	Rat	Review on Reuzel et al., 1994a and Hoymann et al., 1995	NOAEL for inflammatory and other non-neoplastic pulmonary changes: 0.2 mg/m³	Feron et al., 2001	3

1 Reliability key: 1 = method and description are in accordance with test guidelines
2 = falling short of highest standards concerning aspects of protocol or reporting
3 = method or report not in accordance with test guidelines
4 = minimal description of method and report

4.1.2.9 Toxicity for reproduction

4.1.2.9.1 Studies in animals

Effects on fertility

No multigenerational and fertility studies are available for MDI.

The only data available are from (sub) chronic toxicity studies. It has to be stressed that such studies can only give scarce information concerning effects on fertility as the presented data usually concern reproductive organ weights and histopathology but give no information on parameters allowing an evaluation of the reproductive function (e.g. estrus cycle, time to mating, indices of mating, fertility, gestation, birth, pups viability and lactation, sperm number, motility, morphology). However, the outcome of repeat dose studies, if adequately designed and well performed with appropriate examination of the reproductive organs, can be sufficient to fulfil the base set requirement related to the screening of a substance for reproductive (i.e. fertility) toxicity.

Data on gross pathology and histopathology in the reproductive organs of both sexes can be derived from a 24 months chronic inhalation toxicity and carcinogenicity study of respirable polymeric MDI aerosol in Wistar rats, during which 70 rats/sex group (each group subdivided into one satellite group of 10 rats/sex and one main group of 60 rats/sex; exposure of the satellite group was limited to 1 year) had been treated with polymeric MDI at doses of 0, 0.2, 1.0 and 6 mg/m³ respirable polymeric MDI aerosol (93.5% < 4.2 µg) for 6 hours/day, 5 days/week (Reuzel et al., 1994a).

At the end of the study rats were killed, autopsied and examined for gross pathological changes. Several organs (respiratory tract, adrenals, heart, spleen, liver, brain, testes and kidneys) of all rats of the satellite groups and all survivors of the main groups were weighed. In the satellite groups histopathological examination was carried out of a number of organs and of all gross lesions of all rats of the 2-year study. Different organs or tissues (including epididymides, mammary glands, ovaries, prostate, seminal vesicles, testes and uterus) and all gross visible lesions were examined by light microscopy of the low and mid-concentration decedents.

Moreover, in low and mid-concentration survivors of the main groups, respiratory tissues and all gross lesions were subjected to histopathological examination.

No adverse effects were observed on general health, survival and body weight.

In both the satellite and the main groups the absolute and relative organ weights did not show differences between the control groups and the test groups that could be attributed to exposure. However, rats of the low-concentration group showed a statistically higher mean relative testes weight as compared to controls ($p<0.05$) and mean relative testes weight of the other exposure groups was also higher than controls but not to a statistically significant degree. The increases were not concentration-related. The ovaries were not weighed.

Gross pathology revealed discoloured, cystic and granular kidneys, enlarged parathyroids, and frequently occurring atrophic testes in male rats of the main study (8/59 in controls; 5/27, 7/25 and 20/60 in the low, mid and high-concentration groups, respectively) associated with nephrosis which was stated as the main cause of death in males.

In females of the main groups, tumourous masses (17/60, 22/46, 22/43 and 22/58) and secretory activity in mammary glands, ovarian cyst(s) (3/60 in controls, 2/27, 2/18 and 5/59 in exposed groups) and uterine polyps (12/60 in controls; 10/27; 10/18 and 11/59 in low-, mid- and high-concentration groups, respectively) were common findings. Mammary tumours and uterine tumours/polyps were stated as the main cause of death in females. The incidence and distribution of tumours other than lung tumours were not influenced by the substance. The observed lesions in males and females are considered to represent the background pathology of ageing Wistar rats. However, no histological data were presented to corroborate this statement.

The significant increase in testes weight in males at the end of the two-year exposure period were not accompanied by histopathological changes. No concentration-effect relationships were present. Therefore, these higher testes weights were considered by the authors of the study chance findings unrelated to treatment.

The results of a subchronic inhalation toxicity study on pMDI with 6 week old rats, randomly allocated to 4 groups of 30 males and 30 females each, exposed to 0, 4.1, 8.4 and 12.3 mg respirable pMDI/m³ (95% < 5µm) for 6 hours/day, 5 days/week for 13 weeks (followed by a 4 weeks post-treatment) do not indicate an effect on the reproductive organs (Reuzel et al., 1994b).

Histopathological examination was done on collected organs and tissues (including adrenals, epididymides, mammary glands, seminal vesicles, testes and uterus) of 10 rats/sex of the control group and 20 rats/sex of the high-concentration group at the end of the exposure (week 14) and of 10 rats/sex of the control and high-concentration group at the end of the post-treatment period (in week 18).

Gross examination at autopsy did not reveal changes which could be ascribed to treatment. Organ weights and organ-to-body weight ratios were not affected by treatment except for the increased lung relative weight ratios in the mid- and high concentration groups. Treatment-related histopathological and microscopical changes were only found in the respiratory tract in animals at 4.1 mg/m³ and higher. Animals that died or were killed in extremis did not show any other specific pathology than marked thymic involution and lymphoid depletion in the spleen which are considered by the authors of the study to be obviously related to the marked weight loss.

These studies did not reveal clear substance related and/or significant impairment of organs of the reproductive system of the male and the female. Nevertheless, these studies are considered too limited to allow a determination of NOAEL for fertility: none of these studies reported ovaries weights, the changes occurring in the reproductive organs (↑ testes weight, atrophic testes, mammary glands tumourous masses and secretory activity, ovarian cysts, uterine polyps) were considered by the authors as chance findings or as normal background pathology of ageing Wistar rats but no historical control data were presented to corroborate these statements. Finally, it has to be stressed that a potential toxic agent can interrupt the normal function of the reproductive system at any level of the hypothalamic-pituitary-gonads axis, directly at the gonad level or by altering post-gonadal events such as, in males, sperm motility or function or both. The chronic studies did not investigate these functional aspects of reproduction.

A **conclusion (i) on hold** is therefore derived for fertility.

This conclusion is heavily contested by Industry. Industry's position is that this is an end-point that is not of relevance to MDI since toxicity is restricted to the portal of entry (respiratory tract) and reference is made here to acute, subacute and chronic toxicity studies. Furthermore, it was claimed by Industry that the outcome of guideline conform reprotoxicity/fertility/neurotoxicity tests in the rat (not evaluated in-depth by the Rapporteur) with other isocyanates (e.g. toluene diisocyanate and 1,6-hexamethylene diisocyanate) show no effect on reproductive organs, functions and parameters; toxicity of these substances being confined to the respiratory tract with associated clinical symptoms and bodyweight effects (ISOPA, 2003).

Developmental studies

Studies investigating the potential developmental toxicity were conducted for both monomeric MDI and polymeric MDI.

The prenatal toxicity of polymeric MDI (purity: technical product) in pregnant Wistar rats was investigated by aerosol inhalation according to OECD Guideline N° 414 and Directive 87/302/EEC part B, p24 by BASF, 1994 (published in 2000 by Gamer et al.). 25 mated female rats per group were exposed 'whole body' to target concentrations of 0, 1, 4 and 12 mg/m³ in inhalation chambers for 6 hours/day from day 6 to day 15 post coitum (pc). The surviving animals were killed on day 20 pc after a 5-day post-exposure observation period. The study was performed in two replicates comprising about half of the animals each.

Particle size determination of the aerosol yielded MMADs within the respirable range (1.6 µm ≤ MMAD ≤ 2.8 µm). Exposure to 12 mg/m³ of polymeric MDI aerosols caused premature death in 2 out of 25 animals. One animal died during exposure on day 15 pc and a second was found dead in the cage on day 18 pc. These deaths were considered to be substance-related. Beginning on day 12 pc respiratory symptoms developed in some of the animals, which did not recover during the post-exposure observation period. Significant reductions in food and water consumption occurred, which were accompanied by a delay of body weight development. Absolute and relative lung weights were statistically significantly increased. Gross-pathologically, 3 animals showed cachexia and some animals showed dilation of the gastrointestinal tract. No treatment-related findings in dams occurred at concentrations of 1 and 4 mg/m³. The isolated statistically significant decrease in food consumption in the 4 mg/m³ group from days 6 to 9 pc is considered to be incidental.

The sex distribution of the foetuses in all test groups (1, 4 or 12 mg/m³) was comparable with the control foetuses. The differences observed in comparison to the controls were without any biological relevance. The mean placental weights were statistically significantly lower (about

6%) in the high concentration group (12 mg/m³), when compared to the control values. The mean placental weights at 1 and 4 mg/m³ were not influenced by the polymeric MDI exposure. The mean foetal weights were statistically significantly lower (about 10%) in the 12 mg/m³ test group, when compared to the control values. No influences were observed in the 1 and 4 mg/m³ test groups. The external examination of the foetuses revealed malformations in test groups 0, 1 and 12 mg/m³. In the control group, 1 out of 336 examined foetuses (4%) from 1 out of 25 litters (0.3%) showed anophthalmia, while in the 1 mg/m³ test group cleft palate was recorded in 2 out of 338 (0.6%) foetuses (litter incidence: 8.3%). Moreover, anasarca and filiformed tail occurred in 2 foetuses out of 279 (0.7%) from 2 out of 21 litters (9.5%) of the 12mg/m³ group. However, all these malformations are also present in historical control data and are considered to be spontaneous in nature. The external examination of the foetuses revealed no variations in any group. The examination of the organs of the foetuses revealed several types of soft tissue malformations in the foetuses of the 0 and 12 mg/m³ test groups. However, all these soft tissue malformations are also present at a low incidence in historical control data and are considered to be spontaneous in nature. Variations (dilated renal pelvis and/or hydrourether) were detected in all groups with statistically significant and/or biologically relevant differences between the groups. Both findings are very common in the used rat strain and all respective values are fully in the range of biological variation. Various malformations of the sternum, the ribs, and/or the vertebral column were seen in 6 out 175 (3.4%) foetuses (in 4 out of 25 litters = 16%) of the control group, in 11 out of 173 (6.4%) foetuses (in 7 out of 24 litters = 29%) of the 1 mg/m³ group, in 9 out of 177 (5.1%) fetuses (in 8 out of 24 litters = 33%) of the 4 mg/m³ group and in 10 out of 146 (6.8%) fetuses (in 7 out of 21 litters = 33%) of the 12 mg/m³ group. All skeletal malformations recorded appeared without a clear dose-response relationship and are considered to be spontaneous in nature; all aforementioned or very similar skeletal malformations can be found at comparable foetal/litter incidences in the historical control data. The variations elicited were related to the ribs, the sternum, and the clavicula. If assessed separately, each of the skeletal variations recorded appeared without a clear dose-response relationship and/or can be found in a similar frequency in the historical control data. The mean percentage of foetuses/litter with total skeletal variations, however, was statistically significantly increased at the 12 mg/m³ group and the respective value lies above the highest value of the historical control data. Therefore, the increased rate of foetuses per litter with total skeletal variations at 12 mg/m³ might be associated with the exposure. In all groups' signs of skeletal retardation occurred. The statistically significantly increased rate of skeletal retardations at 12 mg/m³ is substance-induced and has to be related to the statistically significantly reduced fetal body weights. The differences between the control and the 1 and 4 mg/m³ test groups in respect to skeletal redardations, however, were considered to be without any biologically relevance.

Thus, the exposure of pregnant Wistar rats in concentrations of 12 mg polymeric MDI/m³ during days 6-15 pc for 6 hours/day resulted in clear signs of maternal toxicity. Maternal toxicity was substantiated by mortality, damage to the respiratory tract, reduced body weight development and reduced mean gravid uterus weights. According to the authors, at this concentration clear signs of developmental (embryo-/foeto-) toxicity in the form of reduced placental and foetal body weights and an increased occurrence of foetal skeletal (and overall) variations and retardations were recorded; however, no substance-induced teratogenic effects were observed up to and including the highest concentration (12 mg/m³).

At concentrations of 1 or 4 mg/m³, no signs of maternal toxicity (at the exception, in the 4 mg/m³ group, of a transient reduction in feed consumption 6-9 pc) and no substance-induced adverse effects on the gestational parameters or the foetuses were recorded.

The authors concluded to the following NOAEL's:

The NOAEL for maternal and developmental toxicity is 4 mg/m³.

The NOAEL for teratogenic effects is ≥ 12 mg/m³.

It is considered by the rapporteur that the NOAEL for maternal toxicity, 4 mg/m³, is based on the mortality, clinical signs, damage to the respiratory tract, decreased bw and decrease in bw gain, decreased feed and water consumption, decreased liver weight, increased lung weight, decreased terminal bw, carcass weight and net weight change and decreased (not statistically significant) gravid uterine weight observed at 12 mg/m^3.

The NOAEL for developmental toxicity is 4 mg/m^3 based on the decreased foetal body weight, on the two dams presenting problems related to their litters (1 fully resorbed litter at termination and 1 dam with 5/15 dead foetuses) observed at 12 mg/m³, on the increase of skeletal variations and retardations and on the increase of total variations (specific skeletal variations/retardations which exhibited significant changes in incidence were: irregularly shaped sternebrae, sternebrae bipartite and thoracic vertebral body incompletely ossified) observed at 12 mg/m^3.

The developmental effects found at 12 mg/m³ indicate that the NOAEL is 4 mg/m³, but these effects can probably be attributed to maternal toxicity. Developmental effects of the highest concern are those that are observed without maternal toxicity.

Table 4.36 Overview of the developmental toxicity test results on inhaled polymeric MDI (Gamer et al., 2000; full report in BASF, 1994)

	0 mg/m³	1 mg/m³	4 mg/m³	12 mg/m³
Litters evaluated	25	24	24	21
Total malformations:				
N° foetus (%)		-	-	-
N° litter (%)		-	-	-
%foetuses/litter		-	-	-↑ °
Total variations:				
N° foetus (%)		-	-	-
N° litter (%)		-	-	-
%foetuses/litter		- ↑ *	- ↑ *	- ↑ * °
External findings:				
External malformations:				
N° foetus (%)				- ↑ * °
N° litter (%)				- ↑ * °
- %foetuses/litter				-
External variations:				
N° foetus (%)				-
N° litter (%)				-
%foetuses/litter				-

Table 4.36 continued overleaf

Table 4.36 continued Overview of the developmental toxicity test results on inhaled polymeric MDI (Gamer et al., 2000; full report in BASF, 1994)

	0 mg/m3	1 mg/m3	4 mg/m3	12 mg/m3
Visceral findings:				
Visceral malformations:				
N° foetus (%)		-		-↑ º
N° litter (%)		-		-
%foetuses/litter		-		-↑ º
Visceral variations:				
N° foetus (%)		-		-
N° litter (%)		-		-
% foetuses/litter		- ↑ *		-
(particularly dilate renal pelvis)				
Skeletal findings:				
Skeletal malformations:				
N° foetus (%)				-
N° litter (%)				-
% foetuses/litter				-
Skeletal variations:				
N° foetus (%)				-↑ º
N° litter (%)				-
% foetuses/litter				- ↑ * º
Skeletal retardations:				
N° foetus (%)				-
N° litter (%)				-
% foetuses/litter				- ↑ * º
Specific skeletal variations/retardations:				
Irregularly shaped sternebrae:				
N° foetus (%)		-		-
N° litter (%)		-		-↑ *
% foetuses/litter		-		-
Sternebrae bipartite:				
N° foetus (%)		-		-
N° litter (%)		- ↑ *		-
% foetuses/litter		- ↑ *		- ↑ *
Thoracic vertebral body incompletely ossified:				
N° foetus (%)		-		- ↑ º
N° litter (%)		-		-
% foetuses/litter		-		- ↑ * º

* statistically significant;
º not within the historical control range
remark: the increased incidence of total foetal variations at exposure concentrations other than 12 mg/m³ were most likely due to the unexpected low number of foetal variations in the concurrent control group and occurred in the absence of a concentration-related relationship therefore they were considered spontaneous in nature.

In a well-conducted developmental range-finding study, according to OECD Guideline N° 414, mated female Wistar rats (8 per group) were exposed 'whole body' to nebulised polymeric MDI aerosol (purity > 95%) by inhalation at exposure levels of 0, 2, 8 and 12 mg/m³ for 6 hours/day

from day 6 up to and including day 15 of pregnancy (Waalkens-Berendsen et al., 1992). On day 21 of pregnancy the female rats were killed and a Caesarian section was performed. The actual concentrations were generally close to the intended concentrations.

No clinical signs or mortality related to treatment were observed during the study. From day 6 to day 9 of pregnancy maternal body weight gain of the 8 and 12 mg/m³ groups was slightly decreased (not statistically significant) when compared with the control group. No differences were observed in body weight, body weight gain, carcass weight and net weight gain from day 0. From day 6 to day 9 of pregnancy the food intake of the 8 and 12 mg/m³ groups was statistically significantly decreased when compared with the control group. No other differences in food consumption were observed. Absolute and relative lung weights were statistically significantly increased in the 12 mg/m³ group. Macroscopically, no treatment-related effects were observed in the females at necropsy.

One animal of the 8 mg/m³ group was not pregnant at Caesarian section on day 21 of pregnancy. All the other animals in all the groups were pregnant. The mean number of corpora lutea, implantation sites, early and late resorptions and consequently the pre- and post-implantation loss showed no statistically significant differences amongst the groups. No statistically significant differences were observed in mean gravid and empty uterus weight, and mean ovary weight.

All the foetuses were alive at Caesarian section. Foetal body weights were comparable in all groups. During the macroscopic examination of the foetuses, one foetus of the control group showed dysmature appearance. One foetus of the 8 mg/m³ and one foetus of the 12 mg/m³ group showed flexed hind paws. In one foetus a subcutaneous haemorrhage in the right hind foot was observed. Furthermore, one foetus of the control group and one foetus of the 2 mg/m³ group showed ringtail. However, none of the observed external abnormalities in the foetuses were considered to be treatment-related.

On the basis of the results obtained in this range-finding study it was concluded by the authors that:

- The NOAEL of polymeric MDI aerosol by inhalation for maternal toxicity was 8 mg/m³ based on the increased lung weights and decreased food intake at 12 mg/m³.
- The NOAEL of polymeric MDI aerosol by inhalation for developmental toxicity was 12 mg/m³.

However, it is considered by the rapporteur that the NOAEL for maternal toxicity is 2 mg/m^3 based on the decreased (statistically significant) food intake (gd 6-9) and decreased (not statistically significant) body weight gain (gd 6-9) observed at 8 mg/m^3. These effects are considered to be treatment-related for the following reasons:

- the statistically significant decrease in food consumption observed at 8 mg/m^3 is higher than 10% (14%) when compared with the control group and is concentration-related: a decrease of 24% as compared with the control group occurs at the same exposure period (gd 6-9) at the higher concentration (12 mg/m^3).
- -the same concentration-related effect (decrease in food consumption) starting at a lower concentration (4 mg/m^3) was also observed at the same gestational period in the main study of Gamer et al. (2000);
- -the toxicological significance of this decrease in food consumption is supported by the concomitant decrease in bw gain. Whereas not statistically significant, important

concentration-related decrease in bw gain was observed (a decrease of 47% as compared with the control group occurs at 8 mg/m^3 and a decrease of 69% as compared with the control group at 12 mg/m^3).

The NOAEL for developmental toxicity is greater than or equal to 12 mg/m^3 based on the lack of treatment-related adverse effects observed at this concentration.

Conclusions on the $NOAEL_{developmental}$ of polymeric MDI

The $NOAEL_{maternal}$ of 2 mg/m³ is the outcome of a range-finding study with pMDI (tested conc: 0, 2, 8 and 12 mg/m³; the NOAEL is based on the statistically significant decrease in food intake and bw gain observed at 8 mg/m³). After evaluation of the overall existing data on polymeric MDI it was concluded that this NOAEL of 2 mg/m³ could be refined by the data of the Gamer study (tested conc: 0, 1, 4 and 12 mg/m³) where an interim exposure regime (4 mg/m³) was tested bringing the $NOAEL_{developmental}$ to 4 mg/m³ (decided because of adverse effects seen at 12 mg/m³, while at the dose of 4 mg/m³ no such effects were seen). In conclusion, the determined $NOAEL_{developmental}$ for polymeric MDI is 4 mg/m³.

Gravid Wistar rats were exposed by whole-body inhalation to clean air (control) and to 1, 3 and 9 mg/m^3 monomeric 4,4'-MDI respectively, for 6 hours/day from days 6 to 15 post coitum (pc) (Buschmann et al., 1994, 1996). Rats were killed on day 20 pc and the following results were obtained: Treatment caused a dose-dependent decrease in food consumption in all substance-treated groups during exposure, returning to normal values after cessation of treatment. The lung weights in the high-dose group were significant increased compared to the sham-treated control animals. Treatment did not influence any other maternal and/or foetal parameters investigated (maternal weight gain, number of corpora lutea, implantation sites, pre- and postimplantation loss, foetal and placental weights, gross and visceral anomalies, degree of ossification), although a slight but significant increase in litters with foetuses displaying asymmetric sternebra(e) was observed after treatment with the highest dose of $9 \cdot 10^{-3}$ mg/l. Although the relevance of an increase of this minor anomaly in doses which cause toxic effects in dams (reduced food consumption, increased lung weights) is limited and the number observed is within the limits of biological variability, a substance-induced effect in the high-dose group cannot be excluded with certainty. Consequently, a no embryotoxic effect level of 3 mg/m^3 was determined in this study by the authors.

Based on the available data on monomeric MDI, the rapporteur concluded that a NOAEL for maternal toxicity cannot be established in this study mainly due to the lack of data concerning the lung (e.g. lung weights were not investigated in the low and medium concentration groups) which is a proved target of MDI.

Table 4.37 Overview of the developmental study results with monomeric MDI (Bushmann et al., 1996)

	0 mg/m³	1 mg/m³	3 mg/m³	9 mg/m³
Litters evaluated	25	26	25	23
Gross external anomalies				
Foetuses evaluated	424	415	403	385
N° foetus (%)	5 (1.18)	7 (1.69)	9 (2.23)	8 (2.08)
N° litter (%)	5 (20)	6 (23.08)	8 (32)	7 (30.43)

Table 4.37 continued overleaf

Table 4.37 continued Overview of the developmental study results with monomeric MDI (Bushmann et al., 1996)

	0 mg/m3	1 mg/m3	3 mg/m3	9 mg/m3
Visceral anomalies				
Foetuses evaluated	203	200	193	186
N° foetus (%)	48 (23.6)	65 (32.5)	51 (26.4)	40 (16.1)
N° litter (%)	18 (72)	23 (88.5)	22 (88)	19 (82.6)
Dilated ureter (slight):				
N° foetus / N° litter	5/5	17*/11	10/7	12/9
Subcutaneous hemorrhages:				
N° foetus / N° litter	1/1	7*/5	3/3	2/1
Skeletal anomalies:				
Foetuses evaluated	221	215	210	199
N° foetus (%)	32 (14.5)	38 (17.7)	29 (13.8)	32 (16.1)
N° litter (%)	15 (60)	16 (61.5)	16 (64)	16 (69.6)
Accessory lumbar ribs:				
N° foetus / N° litter	14/5	7/5	4°*/4	9/5
Asymmetric sternebrae:				
N° foetus / N° litter	5/2	10/7	6/5	11/10*
Incomplete or missing ossification:				
N° foetus (%)	96 (43.4)	86 (40)	76 (36.2)	86 (43.2)
N° litter (%)	25 (100)	25 (96.2)	18°* (72)	22 (95.7)
Nasal:				
N° foetus / N° litter	20/8	12/7	8°*/6	10/6
Sacral vertebral centrae:				
N° foetus / N° litter	7/3	5/3	0°*/0	0°*/0

* $p<0.05$;

° These deviations represent a decrease in the incidence of minor abnormalities and, consequently cannot be considered to be an adverse effect.

For monomeric MDI, the $NOAEL_{developmental}$ is 3 mg/m^3 based on the significant increase in litters with foetuses displaying asymmetric sternebrae after treatment at the highest concentration of 9 mg/m^3.

As the observed adverse effects on the embryonic development may be considered as minor signs of developmental toxicity and as this effect occurs at a monomeric 4,4'-MDI concentration most obviously inducing maternal toxicity (see Section 4.1.2.6.1: the study of Heinrich et al., 1991, mentions that in a 90-day study clear adverse effects on the respiratory tract were seen at 1 mg/m^3 with impact on the general condition of the animals resulting in slightly lower body weight gain, higher lung weight and lower lung functioning), it is considered likely to be secondary to maternal toxicity.

In conclusion, two NOAELs for developmental toxicity can be determined: an NOAEL of 3 g/m^3 for monomeric MDI (Bushmann et al., 1996) and an NOAEL of 4 mg/m^3 for polymeric MDI (Gamer et al., 2000). In the context of this RAR on MDI with CAS-N° 26447-40-5, being a mixture of monomeric and polymeric MDI, the lowest of both NOAELs is chosen to be taken forward for the Risk Characterisation.

4.1.2.9.2 Studies in human

No data are available

4.1.2.9.3 Summary

No fertility or multigenerational studies are available for MDI. Data from (sub)chronic toxicity studies did not reveal clear substance related and/or significant impairment of organs of the reproductive system of the male and female. However, the test protocols of the available (sub)chronic studies present gaps and weaknesses (e.g. not all sex organs were included and systematically examined; lack of control and historical data to put the obtained results into context). In conclusion, the studies are considered too limited to allow a determination of a NOAEL for fertility. As the current database does not adequately cover the toxicity for fertility for MDI, a **conclusion (i)** is reached with regard to fertility. The collection of additional information should, however, not delay the implementation of appropriate control measures needed to address the concerns related to other endpoints (**conclusion (i) on hold**).

Developmental toxicity studies (inhalation) in rats demonstrated signs of developmental toxicity (increased incidence of skeletal variation and slightly impaired body weight gain) at exposure levels that were associated with maternal toxicity during the major period of organogenesis. Such effects are considered likely to be secondary to maternal toxicity and are therefore of limited toxicological significance. In rats, there was no evidence of selective development toxicity at exposure levels that were not associated with maternal toxicity. Consequently, monomeric and polymeric MDI, are not to be considered as developmental toxicants.

The $NOAEL_{(developmental)}$ for monomeric MDI is 3 mg/m³. For polymeric MDI, an $NOAEL_{(maternal)}$ and $NOAEL_{(developmental)}$ of 4 mg/m³ were derived.

The lowest $NOAEL_{(developmental)}$ = 3 mg/m³ will be used for further risk characterisation of the priority substance under study.

There are no data available in humans on fertility or on developmental effects.

No findings indicate any specific developmental effects at exposure levels below those that caused maternal toxicity.

Effects on fertility

Table 4.38 Summary overview of studies on fertility

Substance	Species	Method	Endpoint	Reference	Reliability[1]
Polymeric MDI	Rat	Chronic inhalation study (2 years)	No NOAEL for fertility could be derived	Reuzel et al., 1994a	3
Polymeric MDI	Rat	Subchronic inhalation study (13 weeks)	No NOAEL for fertility could be derived	Reuzel et al., 1994b	3

1 Reliability key: 1 = method and description are in accordance with test guidelines
2 = falling short of highest standards concerning aspects of protocol or reporting
3 = method or report not in accordance with test guidelines
4 = minimal description of method and report

Developmental toxicity

Table 4.39 Summary overview of studies on developmental toxicity

Substance	Species	Method	Endpoint[13]	Reference	Reliability[1]
Monomeric 4,4'-MDI	Rat	OECD Guideline N° 414 0, 1, 3, and 9 mg/m³	NOAEL developmental tox. = 3 mg/m³/day	Buschmann et al., 1994 Bushmann et al, 1996	4 2
Polymeric MDI	Rat	OECD Guideline N° 414 0, 2, 8, and 12 mg/m³	NOAEL mat. tox.: 2 mg/m³/day NOAEL devel. tox.: ≥ 12 mg/m³/day	Waalkens-Berendsen et al.,1992	1
Polymeric MDI	Rat	OECD Guideline N° 414 0, 1, 4, and 12 mg/m³	NOAEL mat. and developmental tox.: 4 mg/m³/day	BASF, 1994 (Gamer et al., 2000)	1

1 Reliability key: 1 = method and description are in accordance with test guidelines
2 = falling short of highest standards concerning aspects of protocol or reporting
3 = method or report not in accordance with test guidelines
4 = minimal description of method and report

4.1.3 Risk characterisation

Humans may be exposed to MDI at the workplace and from use of consumer products.

Both studies in animals and humans are available.

4.1.3.1 General aspects

4.1.3.1.1 Toxico-kinetics, metabolism, distribution

There are few data available on the toxicokinetics and fate of MDI in humans. From biomonitoring studies some absorption of MDI is evidenced by the presence of MDI-associated haemoglobin adducts and urinary metabolites. The half-life of MDA (derived by acid hydrolysis) in urine was determined to be 70-80 hours, the half-life in serum was estimated at 21 days.

No information is available on the toxicokinetics of MDI following oral exposure in animals.

With respect to dermal exposure, contradictory results have been obtained, with none of the studies giving ground for rejection so that it must be concluded that absorption of MDI through the skin must not be neglected. Consequently, a dermal uptake of 1% (Leibold et al., 1998) is used to calculate the body burden in the dermal exposure assessment.

With respect to inhalation exposure, there is good reliable data regarding distribution/excretion in experimental animals. One study indicates that MDI (or a MDI-derived product) is uniformly distributed throughout the organism, with predominance for the lungs, muscle, kidneys and the

[13] specifications mentioned in this summary table are those given by the Rapporteur. For the view of the authors of the studies: see text.

digestive tract. The faecal elimination of MDI and its metabolites is greater than the urinary elimination. These results were confirmed by the results from the full inhalation metabolism / toxicokinetics / distribution study performed by Gledhill (2001a) and published by Gledhill et al. (2005). In this study, approximately 5% of the dose was excreted in urine and 79% in faeces of intact animals. Bile duct cannulated animals excreted approximately 12% of the dose in urine, 14% in bile and 34% of the dose in faeces. Radioactivity was widely distributed with the respiratory and excretory organs containing the highest concentrations of radioactivity. Five metabolites were identified, but no MDA was detected in bile, faeces or urine. In other biomonitoring studies haemoglobin adducts and urine metabolites of MDI were determined. Haemoglobin adducts and urine metabolites of MDI were found in all animals. In another study the transplacental transition of MDI or degradation products (MDX) after MDI inhalation were detected. The MDA analysis, after acid hydrolysis, demonstrated that the highest metabolite levels were measured in the maternal blood, followed by the placenta, foetus and amniotic fluid.

The vigorous acid hydrolysis used in sample preparation for analysis of biomonitoring studies will convert MDI and MDI conjugates to MDA. From the data generated to date, it is possible to state that MDA is not a significant metabolite of inhaled MDI in the rat. The results of the inhalation metabolism / toxicokinetics / distribution study performed by Gledhill (2001b) and published by Gledhill et al. (2005) indicate that a proportion of the MDI dose is converted to metabolites via the intermediary formation of an amine group which is rapidly acetylated. However, it is not possible from the current data to elucidate the steps in the biological transport and transformation of MDI. Glutathione conjugates are considered to play an important role. Further studies using biologically relevant *in vitro* systems are about to commence.

Acute toxicity

Assessment of the available acute toxicity data indicates that inhalation exposure to respirable aerosols of MDI results in toxicity confined predominantly to the respiratory tract. A well-conducted animal study gives a LC_{50} (4 hours, rat) of 490 mg/m^3. Strictly speaking MDI should be classified as toxic by inhalation on the basis of a 4-hour LC_{50} 490 mg/m^3. However, a consensus was reached among European experts (25th ATP, i.e. Dir. 98/8/EC, O.J. 30.12.1998) to consider this value as irrelevant in terms of real-life exposure, because such high values are said not to be achievable except under experimental testing conditions. This pragmatic reasoning is acceptable provided that such high concentrations are indeed never achieved, even through misuse or (further) technological changes in work processes. The available data from the exposure assessment support this concept. Consequently it is proposed to classify MDI as harmful (rather than toxic) by inhalation. In other words, in terms of pure effect assessment MDI is toxic by inhalation. However, if one considers the exposure assessment, it is reasonable to consider MDI as harmful only and to apply the risk management phrase 'harmful by inhalation'.

The limited data available from animal studies indicate that MDI is of low oral and dermal acute toxicity, with an oral LD_{50} (rat) > 10,000mg/kg and dermal LD_{50} (rabbit) > 10,000 mg/kg.

Irritation

Based on both animal studies and human experience, MDI can be stated to be a skin and eye irritant. Based on 'regulatory' acute toxicity studies no conclusions can be drawn regarding the respiratory irritating properties of MDI. However, the repeated dose studies, mechanistic studies, and studies in humans do indicate that MDI causes irritation of the respiratory tract. A RD_{50} (mice) due to pulmonary irritation of 32 mg/m³ was found. A LOEL (rat) of 0.7 mg/m³ was observed related to a transient disturbance of the air/blood barrier function, but without evidence

of cytotoxicity or pulmonary function changes (Pauluhn, 2000). In the opinion of the rapporteur, these transient alterations are indicative of "pulmonary irritation", even if such injuries appear to be rapidly reversible and are of no high concern. Consequently, according to the rapporteur, 0.7 mg/m³ is a LOAEL, situated probably very close to the NOAEL. In a subsequent publication of Pauluhn (2002b), based partly on the same dataset, an acute irritant threshold concentration of 0.5 mg/m³ was estimated for polymeric MDI. As this estimated NOAEL is based on most sensitive endpoints in BALF, the NOAEL of 0.5 mg/m³ is used for further risk characterisation[12]. Based on studies in animals and humans, MDI should, therefore, be classified as an irritant to the skin, the eyes, and the respiratory system.

Sensitisation

MDI has also a skin sensitising potential. Animal studies indicate that MDI is a strong allergen. Human case reports describe allergic contact dermatitis due to MDI exposure.

MDI is a potential respiratory sensitiser in animals and humans. Animal studies have shown that respiratory sensitisation can be induced by skin contact with MDI. The quantitative relationships between exposure (concentration, duration, rate of exposure, route of exposure) and incidence of sensitisation have not been established.

Consequently, at the present time it is not possible to define reliable exposure-response relationships with regard to the risk of sensitisation for MDI (or indeed for any other known respiratory sensitiser). The current knowledge/state of the art in this field does not yet allow taking a decision regarding the existence of a threshold level for sensitisation. Because animal data support the hypothesis that respiratory hypersensitivity may be induced by skin contact and because such possibility has not been excluded in studies involving humans, it is reasonable to consider that it is not only important to reduce inhalation exposure but also to avoid skin contact.

The mechanism behind isocyanate-related hypersensitivity is still obscure. Several publications indicate that complex immunological reactions are involved in the sensitisation process to MDI. Immediate allergic, late allergic and dual-phase responses can occur. Humoral as well as cellular immunity may be involved in the pathogenesis of hypersensitivity due to isocyanates. The specific humoral response can be IgE as well as IgG mediated. Cross-reactivity with other isocyanates has been described in several publications.

Repeated-dose toxicity

No results from repeated-dose toxicity tests are available for the oral and dermal route of exposure.

Well-conducted short-term and long-term inhalation animal studies indicate the respiratory tract to be the target organ of respirable MDI aerosol.

For short-term toxicity (in rats), the most reliable LOAEL found for increased lung weights is 2 mg/m^3, the most reliable NOAEL being 1.4 mg/m³. However, in a more recent mechanistic study (also short-term toxicity in rats) a LOAEL of 1.1 mg/m³ was found for changes in the phospholipid content of alveolar macrophages and non-specific cell proliferation of Type II pneumocytes. Consistent findings were reported in another subacute study (LOAEL of 1 mg/m³). In an acute rat inhalation study a LOAEL of 0.7 mg/m³ was found for transient dysfunction of the pulmonary epithelial barrier, related to a dysfunction of pulmonary surfactant. In a subsequent publication of the same author an acute irritant threshold concentration of 0.5 mg/m³ was estimated. Although these transient alternations are of no high concern, the rapporteur is of

the opinion that it is appropriate and prudent to use this estimated NOAEL in further risk characterisation of short-term toxicity[12].

The most reliable NOAEL for chronic toxicity (in rats) found in these studies, seems to be 0.2 mg/m^3. In a review of the Reuzel et al. (1990, 1994a) and the Hoymann et al. (1995) studies, Feron et al. (2001) concluded that low incidences of lung tumours occurred at the highest dose level in both studies but no lung tumours were found at lower dose levels. For inflammatory and other non-neoplastic pulmonary changes, establishment of a NOAEL of 0.2 mg/m³ (exposure 6 hours/day, 5 days/week for 24 months) was considered scientifically justifiable for both polymeric and monomeric MDI.

The effect of long-term exposure of MDI on the respiratory system of humans has been described in several studies. Long-term exposure to MDI tends to cause restriction of pulmonary function and decline in pulmonary diffusing capacity. In addition to reports of cases of hypersensitivity pneumonitis, pleuritis, and progressive fibrosing alveolitis it may be concluded that chronic exposure to even low levels (but mostly undetermined or below 0.05 mg/m³) of MDI carries a risk of respiratory disease.

For further risk characterisation, a NOAEL = 0.2 mg/m³ (Reuzel et al., 1990, 1994a) will be used for long-term inhalation exposure (workers), the NOAEL = 0.5 mg/m³ (Pauluhn, 2002b) will be used for short-term inhalation exposure (consumers).

Mutagenicity

In our view, tests assessing the mutagenic potential of MDI *in vitro* and *in vivo* provide no convincing evidence of mutagenic and genotoxic activity. The results, from the requested *in vivo* micronucleus test, indicate that aerosolised, inhaled MDI at concentrations as high as 118 mg/m³ air (a concentration high enough to produce portal-of-entry-specific toxic effects, including statistically significantly increased lung weights especially in nose-only exposed rats) did not induce cytogenetic damage *in vivo*.

However, we still require a better knowledge of the rate of *in vivo* conversion of MDI to MDA (or other metabolites) in order to interpret properly the existing data. Pending final results of the ongoing metabolism studies (referred to in Section 4.1.2.1: Toxico-kinetics, metabolism, distribution), no definite firm conclusions on the mutagenic potential of MDI can be drawn. However, at the current stage, the weight of evidence based on experimental data suggests that mutagenicity is of no concern.

The endpoint should be re-assessed once the metabolism studies have been completed.

Carcinogenicity

No carcinogenicity studies are available using the oral or dermal route of exposure.

In a well-conducted chronic toxicity/carcinogenicity animal inhalation study no adverse effect on the distribution and incidence of tumours apart from tumours in the lungs were found (Reuzel et al., 1990, 1994a). In another long-term animal inhalation study a single bronchio-alveolar adenoma was found at 2.05 mg/m^3 MDI (Hoymann et al., 1995).

Although the mechanism for the polymeric MDI-associated lung tumours is not known, Reuzel et al. (1994a), proposed cytotoxicity and the ensuing repair reactions and hyperplasia as a pathogenesis, thus implying that exposure to polymeric MDI at levels which do not result in recurrent tissue damage should not produce tumours.

On the other hand Hoymann et al. (1995) and Sepai et al. (1995) considered that oncogenesis by MDI could be due to its biotransformation to MDA, which is known to be carcinogenic and has been detected, either in acid hydrolysed urine or as haemoglobin adducts, following rat and human exposures to MDI. However technical issues as well as other possible pathways for the formation of haemoglobin adducts (binding to and transport by GSH, followed by solvolysis), possibly weaken this hypothesis. It has to be noted that while such a mechanism is conceivable, there is insufficient evidence currently to clearly attribute any carcinogenic effect to the metabolic formation of MDA. Biotransformation studies are certainly needed to settle the issue. Consequently, there is inadequate evidence of carcinogenicity in humans and limited evidence in experimental animals.

It may be mentioned here that in the U.S. EPA's IRIS updated inhalation reference concentration (RfC) assessment and carcinogenicity assessment for MDI (1998), the inhalation RfC for MDI, 0.0006 mg/m^3, was derived using benchmark concentration (BMC) analysis on basal cell hyperplasia of the olfactory epithelium of chronically-exposed male rats. The RfC is a concentration estimated by the US agency to be a concentration that is likely to be without appreciable risk of deleterious non-cancer effects during a lifetime exposure of the human population and is not part of the workplace scenarios.

In addition, according to the same EPA document, based on U.S.EPA proposed guidelines, the carcinogenic potential of MDI is categorised as "cannot be determined", but there should be suggestive evidence that raises concern for possible carcinogenic effects. This U.S.EPA's assessment is based on the observation that MDA, a known animal carcinogen and reaction product of MDI, has been detected in body fluids of both laboratory animals and humans following MDI exposure. However, the MDA observed was generated by analytical preparative techniques and a recent radiolabel study found no MDA generated *in vivo* in rats after inhalation of MDI.

Reproductive toxicity

There are no data available in humans on fertility or on developmental effects. No fertility or multigenerational animal studies are available for MDI. Data from (sub)chronic toxicity studies did not reveal clear substance related and/or significant impairment of organs of the reproductive system of the male and female. Nevertheless, the studies are considered too limited to allow a determination of a NOAEL for fertility.

Developmental toxicity (inhalation) studies in rats demonstrated some minor signs of developmental toxicity at exposure levels that were associated with maternal toxicity during the major period of organogenesis. Such effects are considered likely to be secondary to maternal toxicity and have therefore limited significance. There was no evidence of selective development toxicity at exposure levels in rats that were not associated with maternal toxicity. As a consequence, monomeric and polymeric MDI are not to be considered as developmental toxicants. Two NOAELs$_{(developmental)}$ can be determined: an NOAEL of 3 mg/m³ for monomeric MDI (Bushmann et al., 1996) and a NOAEL of 4 mg/m³ for polymeric MDI (Gamer et al., 2000). In the context of this RAR on MDI with CAS-N° 26447-40-5, being a mixture of monomeric and polymeric MDI, the lowest of both NOAELs is chosen to be taken forward for the Risk Characterisation.

No findings indicate any specific developmental effects at exposure levels below those that caused maternal toxicity.

4.1.3.2 Workers

Assuming that oral exposure is prevented by personal hygienic measures, the risk characterisation for workers is limited to the dermal and respiratory routes of exposure. Furthermore, it is presumed that adequate risk reduction measures are taken to prevent accidental exposure.

Acute toxicity

Because it is generally known that MDI is a harmful, irritating and sensitising agent, high inhalation and dermal exposure levels are avoided in practice and the use of protective measures have been taken into account in the exposure estimates.

Inhalation

First it has to be mentioned that the artificially generated aerosols required to conduct animal studies are considerably in excess of what is likely to occur in human exposures. Indeed, as reported in the recent ISOPA review (1998) the saturated vapour concentration is not exceeded and hence aerosols cannot form under normal use. While this has to be taken into account, it must be mentioned too that the inherent purpose of toxicity testing using animals is not to reproduce human exposure exactly, but to evaluate the potential of chemicals to cause damage.

The acute inhalation LC_{50} values reported in the literature vary widely. The very low vapour pressure of MDI at ambient temperature makes it very difficult to generate an atmosphere having sufficient concentration to cause any toxic effects. The International Isocyanate Institute Inc. developed a technique to create reproducible atmospheres of respirable aerosols of MDI for inhalation tests. The derived aerosol comprised a >95% respirable fraction. The particle size distribution of 2.1 μm MMAD, having a GSD of 1.6, meets the current international recognised criteria for acute inhalation studies on rats. However, both the aerosol developed and the conditions required to achieve the LC_{50} (4 hours, rat) of 490 mg/m^3 are artificial and not normally experienced in actual handling and use. According to information obtained from industry, typically nozzles in use for MDI-based spray applications (high pressure or airless) have MMADs of 40-120 μm and, moreover, MDI is never present alone but in combination with other compounds, which reduces the concentration of MDI with time. Consequently, in a recent ISOPA review (1998), about the evaluation of acute inhalation toxicity, it is claimed that "There is virtually no overlapping [between experimental aerosols and industrial practice] in the respirable region of the aerosols." In other words, due to the physical properties of these aerosols and the high settling velocity of particles generated under real life conditions, it may be considered that there is presently no potential of exposure to acutely toxic concentrations or doses to such aerosols. Nevertheless, possible changes in technology, either intentional or through misuse, cannot be excluded in the future.

The LC_{50} (4-hour)-value of 490 mg/m^3 is much higher than the estimated short-term inhalation exposure level of 0.1 mg/m^3 for Scenario 1 (chemical industry, MDI and prepolymer production).

Consequently, risk reduction measures, additional to those already taken to prevent accidental exposure, are not indicated for Scenario 1 (**conclusion (ii)**).

The LC_{50} (4-hour)-value of 490 mg/m^3 is also much higher than the estimated short-term inhalation exposure level of 0.1 mg/m^3 for Scenario 2 in general (downstream users, MDI as an intermediate in the industrial and skilled trade sectors). The LC_{50} (4-hour)-value of 490 mg/m^3 is

also much higher than the estimated short-term inhalation exposure level of 0.57 mg/m^3 for specialist contractor foam applicators.

Consequently, risk reduction measures, additional to those already taken to prevent accidental exposure, are not indicated for Scenario 2 (**conclusion (ii)**).

Dermal contact

Because dermal exposure in Scenario 1 (worst case being 650 mg/day corresponding to 9.29 mg/kg bw/day for a 70 kg worker) is low compared to the dermal LD_{50}-value (10,000 mg/kg bw/day), **conclusion (ii)** is also applicable for this scenario.

Dermal exposure in Scenario 2 (worst case being 3,500 mg/day for spraying corresponding to 50 mg/kg bw/day for a 70 kg worker) is low compared to the dermal LD_{50}-value (10,000 mg/kg bw/day); **conclusion (ii)** is also applicable for this scenario.

Table 4.40 Summary of risk characterisation for acute toxicity for workers

Scenario	Route of exposure	LD_{50}, LC_{50}	exposure	MOS	Concern of risk to human health
Scenario 1: Chemical industry	Inhalation	490 mg/m³	0.1 mg/m³	4,900	no
	Dermal	10,000 mg/kg bw	9.29 mg/kg bw	1,076	no
Scenario 2: Downstream users (in general)	Inhalation	490 mg/m³	0.1 mg/m³	4,900	no
	Dermal	10,000 mg/kg bw	18.57 mg/kg bw	539	no
Scenario 2: Specialist contractor foam applicators	Inhalation	490 mg/m³	0.57 mg/m³	860	no
	Dermal	10,000 mg/kg bw	50 mg/kg bw	200	no

Irritation

Skin

Exposure to the skin cannot be excluded in either Scenario 1 or Scenario 2. Giving the irritating action on the skin it is concluded that MDI is of concern for workers with regard to skin effects but there is no need for risk reduction measures beyond those which are being applied already (**conclusion (ii)**), except for unprotected workers on building sites. As on building sites, occupational hygiene standards are often low and PPE might not be worn, **conclusion (iii)** is reached for unprotected workers on building sites: there is a need for limiting the risks on building sites, risk reduction measures which are already being applied shall be taken into account.

Eyes

Exposure to the eyes to liquid MDI is possible incidentally by splashing or due to hand contact with the eyes. Given the irritating action on the eyes it is concluded that MDI is of concern for workers with regard to eye effects but there is no need for risk reduction measures beyond those which are being applied already in both Scenario 1 and Scenario 2 (**conclusion (ii)**), except for unprotected workers on building sites. As on building sites, occupational hygiene standards are often low and PPE might not be worn, **conclusion (iii)** is reached for unprotected workers on

building sites: there is a need for limiting the risks on building sites, risk reduction measures which are already being applied shall be taken into account.

Respiratory tract

Studies in animals as well as humans provide clear evidence of respiratory tract irritation due to MDI. Respiratory tract irritation cannot be excluded in either Scenario 1 or Scenario 2.

Although it is not currently done, a MOS is calculated for respiratory tract irritation. As a starting point the NOAEL of 0.5 mg/m³ for transient dysfunction of the pulmonary epithelial barrier related to a dysfunction of pulmonary surfactant, was used (Pauluhn, 2002b). The occupational reasonable worst case exposure levels were used as the second starting point for the risk characterisation of both scenarios. We consider the derived MOS of 9.4 for Scenario 1 (chemical industry), MOS of 10 for Scenario 2 (downstream users in general: MDI as an intermediate in the industrial and skilled trade sectors) and the MOS of 1.3 for Scenario 2 (specialist contractor foam applicators) insufficient.

For this endpoint, **conclusion (iii)** is reached for all scenarios: there is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Table 4.41 Summary of risk characterisation for respiratory tract irritation for workers

Scenario	Route of exposure	NOAEL	exposure	MOS	Concern of risk to human health
Scenario 1: Chemical industry	Inhalation	0.5 mg/m³	0.053 mg/m³	9.4	yes
Scenario 2: Downstream users (in general)	Inhalation	0.5 mg/m³	0.05 mg/m³	10	yes
Scenario 2: Specialist contractor foam applicators	Inhalation	0.5 mg/m³	0.4 mg/m³	1.3	yes

Sensitisation

Studies in animals as well as humans provide clear evidence of skin and respiratory sensitisation due to MDI.

An interesting hypothesis, to some extent supported by animal experiments, is that respiratory hypersensitivity may be induced by skin contact. It is therefore not only important to reduce inhalation exposure but probably also to avoid skin contact.

However, investigations have shown that some glove materials used do not ensure complete protection, so that relevant dermal exposure and contact sensitisation may be expected even with usage of PPE. It is assumed that in the industrial chemical sector (Scenario 1) and in the further processing industrial sector (Scenario 2), protective gloves will generally be worn in view of the labelling. On the other hand, in the skilled trade sector (also Scenario 2), e.g. on building sites, the ambient standard of hygiene is often low and protective gloves are not always changed or worn.

As already mentioned, the dermal exposure route may be more important for MDI than the respiratory route with regard to respiratory effects. However, real data are lacking for skin exposure and it is doubtful whether EASE estimations are a useful alternative. This is even more problematic in view of the sensitising properties of MDI. However, there is already sufficient information available upon which to base a **conclusion (iii)** for this endpoint for both scenarios: there is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Repeated-dose toxicity, systemic effects, including possible carcinogenicity

Inhalation exposure

Studies in animals as well as humans provide clear evidence that the respiratory tract is the target organ for toxicity. In the long-term animal studies local irritation in the respiratory tract is seen as the main effect.

As a starting point the NOAELs from two separate well conducted chronic animal studies (Reuzel et al., 1990, 1994a; Hoymann et al., 1995) can be used: NOAEL = 0.2 mg/m^3. The occupational reasonable worst case exposure level of 0.053 mg/m^3 is used as the second starting point for the risk characterisation of Scenario 1. The derived Margin of Safety (MOS) for the reasonable worst case estimate is 3.77.

When using the occupational reasonable worst case exposure level of 0.05 mg/m^3 for Scenario 2, the derived MOS is 4. When using the occupational reasonable worst case exposure level of 0.40 mg/m³ for expert specialist contractor foam applicators, the derived MOS is 0.5.

Calculation of MOS values of 0.5, 3.77, and 4 clearly indicates the weakness of comparing directly the NOAEL with exposure. The NOAEL/exposure ratio should be better named Toxic Exposure Ratio (TER), it should be at least 100 – as mentioned in the proceedings of the 1996 ISPRA workshop "Risk assessment: theory and practice" (March 27-28). The Margin of Safety is limited to the fraction of TER which exceeds 100. Therefore, a MOS of 0.5, 3.77 and 4 is not at all "safe".

As at the current exposure levels effects cannot be excluded, it is recommended to re-evaluate the occupational exposure limit values.

In practice, risks are decreased by engineering controls and the use of PPE. On the other hand there is need for further information about the exposure levels in Scenario 1 and Scenario 2, in order to refine the risk assessment.

With the knowledge that chronic exposure to even low levels (but mostly undetermined or below 0.05 mg/m³) of MDI involves a respiratory risk, it is concluded for Scenario 1 and Scenario 2 that there is a need for limiting the risks; risk reduction measures, which are already being applied, shall be taken into account (**conclusion (iii)**).

Dermal exposure

There are no dermal repeated dose toxicity studies available. However, dermal exposure cannot be excluded in both Scenario 1 and Scenario 2. Referring to the risk assessment made for sensitisation (respiratory and skin); the concern for sensitisation by the dermal route will involve a risk reduction strategy during which actual dermal exposures will be investigated more thoroughly. Consequently for this endpoint **conclusion (ii)** is reached for both scenarios: there is

at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Combined exposure

For the calculation of the combined MOS the following data, assumptions and formula are used:

MOS_{comb}= NOAEL (mg/kg/day) / total internal body burden (mg/kg/day)

$NOAEL_{inh}$ (in mg/kg/day) = $NOAEL_{inh}$ (in mg/m³) · (1/bw in kg) · inhaled volume during daily exposure period (in m³/day)

NOAEL = 0.2 mg/m³ (Reuzel et al., 1990, 1994a), daily exposure period being 6 hours/day

Bw rat: 0.3 kg; inhalation volume rat: 0.00024 m³/minute (TNO report V97.520, 1998)

$NOAEL_{inh}$ (mg/kg/day) = (0.2 mg/m³) · (1/0.3 kg) · (0.00024 m³/minute · 360 minutes/day) = 0.0576 mg/kg/day

Total internal body burdens for Scenario 1, Scenario 2 (in general), and Scenario 2 (specialist contractor foam applicators) are 0.10; 0.19; 0.56 mg/kg/day respectively (see Section 4.1.1.2.1. and 4.1.1.2.2.).

MOS Scenario 1 = (0.0576 mg/kg/day) / (0.10 mg/kg/day) = 0.6

MOS Scenario 2 (in general) = (0.0576 mg/kg/day) / (0.19 mg/kg/day) = 0.3

MOS Scenario 2 (specialist contractor foam applicators) = (0.0576 mg/kg/day)/ (0.56 mg/kg/day) = 0.1

The MOS for combined exposure is clearly insufficient to protect the worker due to combined exposure for both scenarios. As **conclusion (iii)** is already applicable for the inhalation exposure route and the concern for sensitisation by the dermal route, **conclusion (iii)** is reached for both scenarios: there is a need for limiting the risks, risk reductions measures, which are already being applied, shall be taken into account.

Mutagenicity

Based on the available and updated data, **conclusion (ii)** is reached with regard to genotoxicity for both scenarios: there is at present no need for further information or testing or risk reduction measures beyond those which are being applied already.

Reproductive toxicity

Fertility

No fertility or multigenerational animal studies are available for MDI, and the data from (sub)chronic toxicity studies are considered too scarce to allow a determination of a NOAEL for fertility. As the current database does not adequately cover the toxicity for fertility for MDI, a **conclusion (i)** is reached with regard to fertility for both scenarios: there is a need for further information and/or testing. The collection of additional information should, however, not delay the implementation of appropriate control measures needed to address the concerns related to other endpoints (**conclusion (i) on hold**).

Developmental toxicity

For the calculation of the MOS, the NOAEL (developmental) = 3 mg/m³/day is used (Buschmann et al., 1996). The occupational reasonable worst case exposure levels used are: 0.053 mg/m^3 (Scenario 1: chemical industry), 0.05 mg/m³ (Scenario 2: intermediate in the industrial and skilled trade sectors), 0.40 mg/m³ (Scenario 2: expert specialist contractor foam sprayers). The derived MOS are given in **Table 4.42**.

For the evaluation of the MOS, a minimal MOS for developmental toxicity is determined. The assessment factors for type of critical effect, dose–response, difference between exposure conditions and exposure pattern, route-to-route extrapolation, confidence of the database, are judged to be 1. According to the ECETOC Technical Report No.68, for intraspecies variation, an assessment factor of 2 can be taken for worker exposure, and for interspecies variation a factor of 1 (for inhalation exposure) multiplied by an additional factor (10: expert judgement) as developmental toxicity is considered here. Taking all assessment factors in consideration, the MOS for developmental toxicity should be ≥ 20. As the MOS for Scenario 1 (chemical industry) and Scenario 2 (downstream users in general) ≥ 20, **conclusion (ii)** is reached with regard to developmental toxicity for these scenarios. Although for expert specialist contractor foam sprayers, the MOS is 7.5, **conclusion (ii)** is reached with regard to developmental toxicity, because the indispensable PPE worn as protection against the irritating effect is found satisfactory enough for the protection against developmental effects.

Table 4.42 Summary of risk characterisation for developmental toxicity for workers

Scenario	Route of exposure	NOAEL (developmental)	Exposure	MOS	Concern of risk to human health
Scenario 1: Chemical industry	inhalation	3 mg/m³	0.053 mg/m³	57	no
Scenario 2: Downstream users (in general)	inhalation	3 mg/m³	0.05 mg/m³	60	no
Scenario 2: Expert specialist contractor foam sprayers	inhalation	3 mg/m³	0.4 mg/m³	7.5, but use of PPE	no

4.1.3.3 Consumers

Assuming the oral exposure is prevented by personal hygienic measures, the risk characterisation for consumers is limited to the dermal and respiratory routes of exposure.

MDI is present in consumer products such as ‘one component foams’ (OCF), paints, putty/filler cartridges, glues, adhesives, hot melt adhesives. Only very few documented measured data are available for consumer exposure. For Scenario 2 (use of OCF) and Scenario 4 (hot melt adhesives) the risk characterisation for inhalation exposure is based on exposure data.

Acute toxicity

Consumer exposure is expected to be acute, i.e. an occasional event of short duration. Because inhalation and dermal exposure for the three scenarios are low compared to the inhalation LC_{50}

and dermal LD_{50}, **conclusion (ii)** is applicable for all scenarios: there is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Table 4.43 Summary of risk characterisation for acute toxicity for consumers

Scenario	Route of exposure	LD_{50}, LC_{50},	Exposure	MOS	Concern of risk to human health
Spray painting (liquid roof coating)	Dermal	10,000 mg/kg bw	0.076mg/kg bw	131,579	no
Use of OCF	inhalation	490 mg/m³	0.0061 mg/m³	80,328	no
	dermal	10,000 mg/kg bw	7.6 mg/kg bw	1,316	no
Glueing, painting, use of putty/filler cartridge	dermal	10,000 mg/kg bw	42 mg/kg bw	238	no
Hot melt adhesives	inhalation	490 mg/m³	0.025 mg/m³	19,600	no
	dermal	10,000 mg/kg bw	2.4 mg/kg bw	4,167	no

Irritation

Skin and eye irritation from direct skin and eye contact during spray painting (Scenario 1), use of OCF (Scenario 2), during glueing, painting or using a putty/filler cartridge (Scenario 3) or during the use of a hot melt adhesive (Scenario 4), cannot be excluded. As for unprotected workers on building sites, consumers probably will not always wear PPE during the use of MDI-containing products. **Conclusion (iii)** is reached for all consumer scenarios for both skin and eye irritation: there is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Respiratory tract irritation cannot be excluded during the use of OCF (Scenario 2) and during the use of a hot melt adhesive (Scenario 4). Assuming a short-term inhalation exposure level of 0.0061 mg/m³ for the Scenario 2 and 0.025 mg/m³ for Scenario 4, and the NOAEL of 0.5 mg/m³ for acute pulmonary irritation (Pauluhn, 2002b), a MOS of 0.5/0.0061 = 82 is achieved for Scenario 2 and a MOS of 0.5/0.025 = 20 is achieved for Scenario 4. As for unprotected workers on building sites, consumers probably will not always wear PPE during the use of MDI-containing products. **Conclusion (iii)** is reached for consumer Scenario 2 and 4 for respiratory tract irritation: there is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account. As Scenario 1 (spray painting: liquid roof coating) and 3 (glueing, painting, using a putty/filler cartridge) causes no (or negligible) inhalation exposure **conclusion (ii)** is applicable for Scenario 1 and 3: there is at present no need for further information and/or testing and for risk reduction measures beyond those which are being applied already.

Sensitisation

As respiratory hypersensitivity may be induced by skin contact, respiratory and skin sensitisation due to MDI, cannot be excluded during spray painting (Scenario 1), the use of OCF (Scenario 2), during glueing or using a putty/filler cartridge (Scenario 3) or during the use of a hot melt adhesive (Scenario 4). However, there is already sufficient information available upon which to base a **conclusion (iii)** for this endpoint for all scenarios: there is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account. (Specific attention should be paid to the situation where a subject has an occupationally acquired sensitisation to MDI).

Lung effects induced by short-term repeated exposure

As consumer exposure is expected to occur occasionally for short-term events, a risk characterisation was made for lung effects induced by short-term exposure for Scenario 2 and 4.

Table 4.44 Risk characterisation for lung effects induced by short-term exposure for consumers

Scenario	Route of exposure	Lung effects induced by short-term exposure (NOAEL from Pauluhn 2002b)	Exposure	MOS	Concern of risk to human health
Use of OCF	inhalation	0.5 mg/m³	0.0061 mg/m³	82	yes
Hot melt adhesives	Inhalation	0.5 mg/m³	0.025 mg/m³	20	yes

As specific attention should be paid to the situation where a subject has an occupationally acquired sensitisation to MDI, **Conclusion (iii)** is reached for Scenario 2 and Scenario 4: values of MOS of 82 and 20 are considered insufficient.

Chronic toxicity/Carcinogenicity

Chronic toxicity from the use of consumer products containing MDI is considered of less concern as consumer exposure of the identified products is expected to occur on occasional events of short duration. For chronic toxicity and carcinogenicity, **conclusion (ii)** is reached for all scenarios: there is at present no need for further information or testing or risk reduction measures beyond those which are being applied already.

Mutagenicity

Based on the available and updated data, **conclusion (ii)** is reached with regard to genotoxicity for all scenarios: there is at present no need for further information or testing or risk reduction measures beyond those which are being applied already.

Reproductive toxicity

Fertility

As the current database does not adequately cover the toxicity for fertility for MDI, a **conclusion (i)** is reached with regard to fertility for all consumer scenarios: there is a need for further information and/or testing. The collection of additional information should, however, not delay the implementation of appropriate control measures needed to address the concerns related to other endpoints (**conclusion (i) on hold**).

Developmental toxicity

For the calculation of the MOS, the NOAEL (developmental) = 3 mg/m³/day is used (Buschmann et al., 1996). The reasonable worst case exposure level used are: 0.0061 mg/m^3 (Scenario 2: use of OCF), 0.025 mg/m³ (Scenario 4: use of hot melt adhesives). The derived MOS are put in **Table 4.45**.

Table 4.45 Summary of risk characterisation for developmental toxicity for consumers

Scenario	Route of exposure	NOAEL (developmental)	Exposure	MOS	Concern of risk to human health
Use of OCF	inhalation	3 mg/m³	0.0061 mg/m³	492	no
Hot melt adhesives	inhalation	3 mg/m³	0.025 mg/m³	120	no

For the evaluation of the MOS, a minimal MOS for developmental toxicity is determined. The assessment factors for type of critical effect, dose–response, difference between exposure conditions and exposure pattern, route-to-route extrapolation, confidence of the database, are judged to be 1. According to the ECETOC Technical Report No.68, for intraspecies variation, an assessment factor of 3 can be taken for the general population, and for interspecies variation a factor of 1 (for inhalation exposure) multiplied with an additional factor (10: expert judgement) as developmental toxicity is considered here. Taking all assessment factors in consideration, the MOS for developmental toxicity should be ≥ 30. As the MOS for all consumer scenarios ≥ 30, **conclusion (ii)** is reached with regard to developmental toxicity for all scenarios: there is at present no need for further information or testing or risk reduction measures beyond those which are being applied already.

4.1.3.4 Humans exposed via the environment

On the basis of the estimated exposure of humans to MDI through the environment and the effect data on mammals, EUSES models provides margin of safety factors (MOS) as shown in **Table 4.46**:

Table 4.46 Margin of safety factors (MOS) calculated with EUSES for MDI

	MOS Total	MOS Air
Local scale:		
Production	$1.32\ 10^4$	$3.28\ 10^5$
Processing to polyurethanes	$2.24\ 10^3$	$1.13\ 10^4$
Processing of prepolymers – speciality MDI'S	$6.75\ 10^3$	$5.05\ 10^4$
Processing of prepolymers – other than speciality MDI'S	$4.27\ 10^3$	$2.54\ 10^4$
Processing to prepolymers	$5.33\ 10^3$	$3.46\ 10^4$
Regional scale	$1.52\ 10^4$	$1.30\ 10^6$

MOS_{Air} are the ratio between the inhalatory NOAEL (0.2 mg/m^3) and the daily doses through air.

MOS_{Total} are the ratio between the inhalatory NOAEL (0.2 mg/m³) expressed in mg/kg.day (0.0576 mg/kg.day, see Section 4.1.3.2) and the total daily doses.

Nevertheless, given that exposure levels are most probably much lower than calculated by the model on account of the very high reactivity of MDI with water and the worst case assumptions upon which the calculations were based (see Section 3.1), and in view of the MOS obtained (minimum 2,240), it can be concluded that exposure of humans to MDI through the general environment is not expected to lead to any health hazard. Human exposure to MDI indirectly via

the environment is of no concern. **Conclusion (ii)** is reached, there is at present no need for further information or testing or risk reduction measures beyond those which are being applied already.

Combined exposure

Combining occupational, consumer and indirectly via the environment exposure will not materially influence the characterisation of the risks associated with occupational exposure alone (see Section 4.1.3.2).

4.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

With regard to the physico-chemical properties and with regard to the occupational, consumer, indirect and combined exposure described in Section 4.1.1.2, 4.1.1.3, 4.1.1.4 and 4.1.1.5, MDI is not expected to cause specific concern relevant to human health.

5 RESULTS

5.1 ENVIRONMENT

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

5.2 HUMAN HEALTH

5.2.1 Human health (toxicity)

5.2.1.1 Workers

Conclusion (i) There is need for further information and/or testing.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (i) on hold is reached because:

- The current database does not adequately cover the toxicity for fertility. The collection of additional information should, however, not delay the implementation of appropriate control measures needed to address the concerns related to other endpoints (**conclusion (i) on hold**).

Conclusion (iii) is reached because:

- Health risks due to occupational exposure cannot be excluded with regard to irritation, both skin and eyes, for unprotected workers on building sites.
- Health risks due to occupational exposure cannot be excluded with regard to respiratory tract irritation.
- Health risks due to occupational exposure cannot be excluded with regard to sensitisation (dermal contact and inhalation exposure).
- Health risks due to occupational exposure cannot be excluded with regard to repeated inhalation exposure.

Table 5.1 Overview of conclusions with respect to occupational risk characterisation

End point and outcome of the key study used	Conclusions valid for the occupational scenarios			
in risk characterisation (between brackets)	Scenario 1: Chemical Industry		Scenario 2: Downstream users	
	MOS	Conclusion	MOS	Conclusion
Acute toxicity				
- oral (LD_{50} rat >10,000 mg/kg bw)	-	ii	-	ii
- dermal (LD_{50} rabbit >10,000 mg/kg bw)	1,076	ii	-539 (200 for specialist contractor foam applicators)	ii
- inhalation (LC_{50}, 4 hours, rat: 490 mg/m3)	4,900	ii	4,900 (860 for specialist contractor foam applicators)	ii
Irritation				
dermal (irritant)	-	ii	-	ii (exception: unprotected workers on building sites: iii)
eyes (irritant)	-	ii	-	ii (exception: unprotected workers on building sites: iii)
respiratory tract	9.4	iii	10 1.3 (specialist contractor foam applicators)	iii iii
Sensitisation				
- dermal (sensitising)	-	iii	-	iii
- inhalation (sensitising)	-	iii	-	iii
Repeated dose toxicity, systemic effects, including possible carcinogenicity				
- dermal (no studies)	-	ii	-	ii
- inhalation (NOAEL rat, 0.2mg/m^3)	3.77	iii	4	iii
- combined	0.6	iii	0.3 0.1 (specialist contractor foam applicators)	iii iii
Mutagenicity (no evidence for mutagenicity)	-	ii	-	ii
Reproductive toxicity				
- inhalation				
Fertility (database not adequately enough)	-	i on hold	-	i on hold
Developmental toxicity ($NOAEL_{dev}$, rat, 3 mg/m^3/d)	57	ii	60 7.5 (specialist contractor foam applicators), but PPE	ii ii

5.2.1.2 Consumers

Conclusion (i) There is need for further information and/or testing.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (i) on hold is reached because:

- The current database does not adequately cover the toxicity for fertility. The collection of additional information should, however, not delay the implementation of appropriate control measures needed to address the concerns related to other endpoints (**conclusion (i) on hold**).

Conclusion (iii) is reached because:

- Risk reduction measures should be considered that will ensure protection of consumers from eye and skin and respiratory tract irritation, respiratory and skin sensitisation, and lung effects induced by short-term repeated exposure.

Table 5.2 Overview of conclusions with respect to consumer risk characterisation

Endpoint	Conclusions valid for the consumer scenarios			
	Scenario 1	Scenario 2	Scenario 3	Scenario 4
Acute toxicity (oral, dermal, inhalation)	ii	ii	ii	ii
Irritation (skin, eyes)	iii	iii	iii	iii
Irritation (respiratory tract)	ii	iii	ii	iii
Sensitisation (dermal, inhalation)	iii	iii	iii	iii
Lung effects induced by short-term repeated exposure	ii	iii	ii	iii
Chronic toxicity / carcinogenicity	ii	ii	ii	ii
Mutagenicity	ii	ii	ii	ii
Reproductive toxicity (fertility)	i on hold	i on hold	i on hold	i on hold
Reproductive toxicity (developmental)	ii	ii	ii	ii
Physicochemical properties	Ii	ii	ii	ii

5.2.1.3 Humans exposed via the environment

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

5.2.1.4 Combined exposure: workers/consumers/indirectly via the environment

Conclusion (i) There is need for further information and/or testing.

Conclusion (iii) There is a need for limiting the risks; risk reduction measures which are already being applied shall be taken into account.

Conclusion (i) on hold is reached because:

- The current database does not adequately cover the toxicity for fertility of MDI. The collection of additional information should, however, not delay the implementation of

appropriate control measures needed to address the concerns related to other endpoints (**conclusion (i) on hold**).

Conclusion (iii) is reached because:

- Health risks due to combined occupational and consumer exposure cannot be excluded with regard to irritation (eyes, skin, respiratory tract).
- Health risks due to combined occupational and consumer exposure cannot be excluded with regard to sensitisation (dermal contact and inhalation exposure).
- Health risks due to combined occupational and consumer exposure cannot be excluded with regard to repeated inhalation exposure.

5.2.2 HUMAN HEALTH (PHYSICO-CHEMICAL PROPERTIES)

Conclusion (ii) There is at present no need for further information and/or testing or for risk reduction measures beyond those which are being applied already.

Conclusion (ii) is reached because:

- No concern is expected related to the physico-chemical properties of the substance for human populations (occupational, consumer and humans exposed via the environment).

6 REFERENCES

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ABBREVIATIONS

ADI	Acceptable Daily Intake
AF	Assessment Factor
ASTM	American Society for Testing and Materials
ATP	Adaptation to Technical Progress
AUC	Area Under The Curve
B	Bioaccumulation
BBA	Biologische Bundesanstalt für Land- und Forstwirtschaft
BCF	Bioconcentration Factor
BMC	Benchmark Concentration
BMD	Benchmark Dose
BMF	Biomagnification Factor
BOD	Biochemical Oxygen Demand
bw	body weight / *Bw*, *bw*
C	Corrosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
CA	Chromosome Aberration
CA	Competent Authority
CAS	Chemical Abstract Services
CEC	Commission of the European Communities
CEN	European Standards Organisation / European Committee for Normalisation
CEPE	European Committee for Paints and Inks
CMR	Carcinogenic, Mutagenic and toxic to Reproduction
CNS	Central Nervous System
COD	Chemical Oxygen Demand
CSTEE	Scientific Committee for Toxicity, Ecotoxicity and the Environment (DG SANCO)
CT_{50}	Clearance Time, elimination or depuration expressed as half-life
d.wt	dry weight / dw
dfi	daily food intake
DG	Directorate General
DIN	Deutsche Industrie Norm (German norm)
DNA	DeoxyriboNucleic Acid
DOC	Dissolved Organic Carbon
DT50	Degradation half-life or period required for 50 percent dissipation / degradation
DT90	Period required for 90 percent dissipation / degradation
E	Explosive (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
EASE	Estimation and Assessment of Substance Exposure Physico-chemical properties [Model]

EbC50	Effect Concentration measured as 50% reduction in biomass growth in algae tests
EC	European Communities
EC10	Effect Concentration measured as 10% effect
EC50	median Effect Concentration
ECB	European Chemicals Bureau
ECETOC	European Centre for Ecotoxicology and Toxicology of Chemicals
ECVAM	European Centre for the Validation of Alternative Methods
EDC	Endocrine Disrupting Chemical
EEC	European Economic Communities
EINECS	European Inventory of Existing Commercial Chemical Substances
ELINCS	European List of New Chemical Substances
EN	European Norm
EPA	Environmental Protection Agency (USA)
ErC50	Effect Concentration measured as 50% reduction in growth rate in algae tests
ESD	Emission Scenario Document
EU	European Union
EUSES	European Union System for the Evaluation of Substances [software tool in support of the Technical Guidance Document on risk assessment]
F(+)	(Highly) flammable (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
FAO	Food and Agriculture Organisation of the United Nations
FELS	Fish Early Life Stage
foc	Organic carbon factor (compartment depending)
GLP	Good Laboratory Practice
HEDSET	EC/OECD Harmonised Electronic Data Set (for data collection of existing substances)
HELCOM	Helsinki Commission -Baltic Marine Environment Protection Commission
HPLC	High Pressure Liquid Chromatography
HPVC	High Production Volume Chemical (> 1000 tonnes/annum)
IARC	International Agency for Research on Cancer
IC	Industrial Category
IC50	median Immobilisation Concentration or median Inhibitory Concentration
ILO	International Labour Organisation
IPCS	International Programme on Chemical Safety
ISO	International Organisation for Standardisation
IUCLID	International Uniform Chemical Information Database [software tool]
IUPAC	International Union for Pure and Applied Chemistry
JEFCA	Joint FAO/WHO Expert Committee on Food Additives
JMPR	Joint FAO/WHO Meeting on Pesticide Residues

Koc	organic carbon normalised distribution coefficient
Kow	octanol/water partition coefficient
Kp	solids-water partition coefficient
L(E)C50	median Lethal (Effect) Concentration
LAEL	Lowest Adverse Effect Level
LC50	median Lethal Concentration
LD50	median Lethal Dose
LEV	Local Exhaust Ventilation
LLNA	Local Lymph Node Assay
LOAEL	Lowest Observed Adverse Effect Level
LOEC	Lowest Observed Effect Concentration
LOED	Lowest Observed Effect Dose
LOEL	Lowest Observed Effect Level
MAC	Maximum Allowable Concentration
MATC	Maximum Acceptable Toxic Concentration
MC	Main Category
MITI	Ministry of International Trade and Industry, Japan
MOE	Margin of Exposure
MOS	Margin of Safety
MW	Molecular Weight
N	Dangerous for the environment (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC
NAEL	No Adverse Effect Level
NOAEL	No Observed Adverse Effect Level
NOEL	No Observed Effect Level
NOEC	No Observed Effect Concentration
NTP	National Toxicology Program (USA)
O	Oxidising (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
OC	Organic Carbon content
OECD	Organisation for Economic Cooperation and Development
OEL	Occupational Exposure Limit
OJ	Official Journal
OSPAR	Oslo and Paris Convention for the protection of the marine environment of the Northeast Atlantic
P	Persistent
PBT	Persistent, Bioaccumulative and Toxic
PBPK	Physiologically Based PharmacoKinetic modelling

PBTK	Physiologically Based ToxicoKinetic modelling
PEC	Predicted Environmental Concentration
pH	logarithm (to the base 10) (of the hydrogen ion concentration $\{H^+\}$
pKa	logarithm (to the base 10) of the acid dissociation constant
pKb	logarithm (to the base 10) of the base dissociation constant
PNEC	Predicted No Effect Concentration
POP	Persistent Organic Pollutant
PPE	Personal Protective Equipment
QSAR	(Quantitative) Structure-Activity Relationship
R phrases	Risk phrases according to Annex III of Directive 67/548/EEC
RAR	Risk Assessment Report
RC	Risk Characterisation
RfC	Reference Concentration
RfD	Reference Dose
RNA	RiboNucleic Acid
RPE	Respiratory Protective Equipment
RWC	Reasonable Worst-Case
S phrases	Safety phrases according to Annex IV of Directive 67/548/EEC
SAR	Structure-Activity Relationships
SBR	Standardised birth ratio
SCE	Sister Chromatic Exchange
SDS	Safety Data Sheet
SETAC	Society of Environmental Toxicology And Chemistry
SNIF	Summary Notification Interchange Format (new substances)
SSD	Species Sensitivity Distribution
STP	Sewage Treatment Plant
T(+)	(Very) Toxic (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
TDI	Tolerable Daily Intake
TG	Test Guideline
TGD	Technical Guidance Document
TNsG	Technical Notes for Guidance (for Biocides)
TNO	The Netherlands Organisation for Applied Scientific Research
ThOD	Theoritical Oxygen Demand
UC	Use Category
UDS	Unscheduled DNA Synthesis
UN	United Nations
UNEP	United Nations Environment Programme

US EPA	Environmental Protection Agency, USA
UV	Ultraviolet Region of Spectrum
UVCB	Unknown or Variable composition, Complex reaction products of Biological material
vB	very Bioaccumulative
VOC	Volatile Organic Compound
vP	very Persistent
vPvB	very Persistent and very Bioaccumulative
v/v	volume per volume ratio
w/w	weight per weight ratio
WHO	World Health Organisation
WWTP	Waste Water Treatment Plant
Xn	Harmful (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)
Xi	Irritant (Symbols and indications of danger for dangerous substances and preparations according to Annex II of Directive 67/548/EEC)

European Commission

EUR 22104 EN **European Union Risk Assessment Report methylenediphenyl diisocyanate (MDI), Volume 59**

Editors: S.J. Munn, R. Allanou, K. Aschberger, O. Cosgrove, S. Pakalin, A. Paya-Perez, G. Pellegrini, B. Schwarz-Schulz, S. Vegro.

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Environment and quality of life series

The report provides the comprehensive risk assessment of the substance Methylenediphenyl diisocyanate (MDI). It has been prepared by Belgium in the frame of Council Regulation (EEC) No. 793/93 on the evaluation and control of the risks of existing substances, following the principles for assessment of the risks to humans and the environment, laid down in Commission Regulation (EC) No. 1488/94.

Part I - Environment

This part of the evaluation considers the emissions and the resulting exposure to the environment in all life cycle steps. Following the exposure assessment, the environmental risk characterisation for each protection goal in the aquatic, terrestrial and atmospheric compartment has been determined.

The environmental risk assessment concludes that there is no concern.

Part II – Human Health

This part of the evaluation considers the emissions and the resulting exposure to human populations in all life cycle steps. The scenarios for occupational exposure, consumer exposure and humans exposed via the environment have been examined and the possible risks have been identified.

The human health risk assessment concludes that there is concern for workers and consumers with regard to irritation of skin, eye and respiratory tract, skin sensitisation and lung effects induced by repeated inhalation exposure.
There is a need for further information and for testing (on hold) on the toxicity for fertility for workers and consumers.
For humans exposed via the environment and for human health (physico-chemical properties) there is no concern.

The conclusions of this report will lead to risk reduction measures to be proposed by the Commission's committee on risk reduction strategies set up in support of Council Regulation (EEC) N. 793/93.

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European Commission – Joint Research Centre
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European Chemicals Bureau (ECB)

European Union Risk Assessment Report

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