4,4′-Methylene diphenyl diisocyanate (MDI)

[101-68-8]

and "polymeric" MDI (PMDI)

[9016-87-9]

Supplement 2008

	MDI	"polymeric" MDI
MAK value	**0.05 mg/m³ I (inhalable fraction) (1992)**	**0.05 mg/m³ I (inhalable fraction) (2007)**
Peak limitation	**Category I, excursion factor 1**	**Category I, excursion factor 1**
	momentary value 0.1 mg/m³ (2000)	**momentary value 0.1 mg/m³ (2007)**
Absorption through the skin	**H (2007)**	**H (2007)**
Sensitization	**Sah (1984, 1992)**	**Sah (1995)**
Carcinogenicity	**Category 4 (2007)**	**Category 4 (2007)**
Prenatal toxicity	**Pregnancy Risk Group C (2007)**	**Pregnancy Risk Group C (2007)**
Germ cell mutagenicity	**–**	**–**
BLW	**10 µg 4,4′-diaminodiphenyl methane per litre urine (2006)**	**–**

Since the last documentation was published in 1992 (documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997), followed by the supplements of 1995 (supplement "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 2000) and 2000 (supplement "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 2000, appearing with this documentation), numerous other studies, including mechanistic studies of various end points of the toxic effects of 4-4′-methylene diphenyl diisocyanate (MDI)

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and "polymeric MDI" (PMDI) have been carried out. Most of the data have been presented and evaluated in detail in the EU Risk Assessment Report (EU 2005). In the following, therefore, only those studies relevant to the evaluation are described in greater detail. The remaining toxicological end points are merely summarized here on the basis of the most recent MAK documentation or the Risk Assessment Report.

MDI occurs in the form of monomeric ("binuclear"), oligomeric ("trinuclear") and polymeric ("polynuclear") MDI (see Figure 1); polymeric MDI usually contains between 30% and 80% monomeric MDI. No toxicological data are available for monomer-free PMDI.

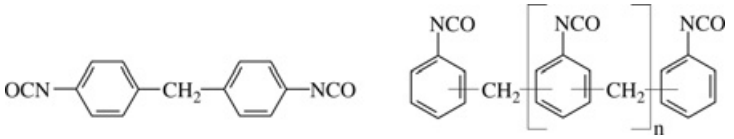

Figure 1 Idealized structures of monomeric, oligomeric (n = 1) and polymeric (n = 2–4) MDI

Monomeric, oligomeric and polymeric MDI contain isocyanate(NCO) groups, which bind covalently to nucleophilic biomolecules with NH_2, OH or SH groups and thus determine the reactivity. It is therefore justified to consider the local effects of monomeric, oligomeric and polymeric MDI together. As MDI has two and PMDI at least three functional groups, numerous reactions can occur up to polymerization. It must be assumed, however, that the reactivity with nucleophilic biomolecules decreases as the degree of polymerization increases. Thus MDI is more likely to undergo addition reactions with low-molecular nucleophils (for example with glutathione). Experimental studies with rats have also confirmed that the systemic availability of MDI is greater than that of oligomeric MDI or PMDI (Pauluhn 2002 a; Pauluhn et al. 2006).

1 Toxic Effects and Mode of Action

As already presented in the documentation from 1992 (documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997), the main effects of MDI in both animal experiments and in humans are irritation and sensitization of the airways.

In animal studies, the earliest indication of irritation observed in the alveoli was an increase in the concentration of total protein (protein exudation) after single exposures to inhalable PMDI aerosol for 6 hours in concentrations of 0.7 mg/m^3 and above (Pauluhn 2000, 2002 a, b). Such effects are attributed to the transient destabilization of the surfactant and are not regarded as adverse because of the rapid reversibility of increased protein exudation. Relevant changes in the

bronchoalveolar lavage (BAL) are not observed after short-term exposure until higher concentrations of PMDI of 2.4 mg/m^3 and above. Clinical symptoms are not observed until 8 mg/m^3. The first signs of irritation are observed at PMDI concentrations of 1 mg/m^3 after repeated exposure for 6 hours a day. In a long-term inhalation study with rats, PMDI aerosol caused chronic inflammatory changes with an increased incidence of bronchioalveolar adenomas and one carcinoma, principally in the lower respiratory tract, after exposure for 6 hours a day to concentrations of 6 mg/m^3. In a long-term inhalation study with MDI aerosol, one pulmonary adenoma was observed in addition to chronic inflammatory changes in the respiratory tract after exposure for 17 hours a day to 2 mg/m^3; 2 mg/m^3 over 17 hours corresponds to exposure to more than 6 mg/m^3 over 6 hours. These findings are regarded as typical sequel reactions of the rat lung to direct alveolar irritation and the associated chronic inflammation (regenerative cell proliferation). There is no evidence of systemic toxicity.

These irritating effects are to be distinguished from allergic bronchial hyperreactivity, (di-)isocyanate asthma, or alveolar hyperreactivity with or without evidence of immunological parameters ("sensitization"). In humans, intensive skin contact with MDI is involved in the induction of specific hyperreactivity of the airways (see documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997). In animal studies, a skin-sensitizing effect (type IV) has been found for MDI but not for PMDI. MDI-induced contact dermatitis has been reported. There are no data available for PMDI. After inhalation exposure, MDI reacts with the nucleophilic lower and higher-molecular components of the liquid film coating the airways, particularly with glutathione (GSH). The low-molecular adducts and conjugates of MDI are absorbed. In contrast, less soluble polymerized adducts and conjugates are subject to mechanical clearance. The metabolism of MDI by hydrolysis and degradation to form 4,4′-diaminodiphenylmethane (MDA)—which was found to be carcinogenic in animal experiments after oral administration—has been postulated, but could not be demonstrated using different experimental models. Merely the formation of acetylated MDA has been observed. The available findings suggest, however, that acetylation occurs at the level of the GSH adduct.

Only some of the studies available to date of the mutagenicity of both monomeric MDI and PMDI can be included in the evaluation. The choice of solvent and the method of adding the dissolved MDI to the aqueous test medium have a decisive influence on the test results as a result of solvolytic effects. A valid micronucleus test yielded negative results in vivo after the inhalation of MDI in concentrations that cause pulmonary irritation. The binding of MDI to DNA in the lungs (the target organ of MDI toxicity) was not observed after long-term inhalation exposure.

The available studies do not provide any evidence of embryotoxic effects of MDI and PMDI. Slight embryotoxic effects were observed in the maternally toxic range of 9 mg/m^3 for MDI and 12 mg/m^3 for PMDI.

2 Mechanism of Action

The reactive NCO group is common to all isocyanates, but it is mainly the different physico-chemical properties of the isocyanates that determine where they are deposited and thus the site of damage in the respiratory tract. Also the ways in which the corresponding biological structure reacts to the damage produced can differ quantitatively within the common spectrum of effects of the different classes of isocyanates (aromatic, aliphatic or alicyclic mono, di or polyisocyanates).

The effects of MDI are based also on the reactivity of the NCO groups. Both the local irritation and the property of MDI to form haptens, with subsequent immunological sequel reactions, is attributed to this. Correspondingly, MDI has the potential to cause both skin and eye irritation and sensitization (asthmogenic potential).

Inhaled diisocyanates are deposited in the respiratory tract where they react with nucleophils. In the lungs, glutathione (GSH) is quantitatively the most important nucleophil (Cantin et al. 1987; Rahman et al. 1999). MDI forms GSH mono-conjugates or GSH bis-conjugates. Where there is an excess of GSH, as in the respiratory tract, mainly the bis-conjugate is formed (Pauluhn et al. 2006). The GSH concentration in the liquid film coating the airways in humans is approximately 200 to 300 µmol/l (Kelly 1999) or 429 µmol/l (Cantin et al. 1987). The GSH level in the plasma, on the other hand, is much lower, with concentrations of < 5 µmol/l (Kelly 1999) or 3 µmol/l (Cantin et al. 1987). More than 95% of the total glutathione is present in the form of GSH (Cantin et al. 1987). The GSH concentration in the erythrocytes was found to be 2.2 mmol/l and thus about 700 times higher than that in the plasma (Bocci 2006). Thus, the erythrocytes in the lung form a GSH reservoir. Normalized to the protein content in the bronchoalveolar lavage fluid the amount of GSH in humans is almost twice as high as that in the rat (Slade et al. 1993). The heterogeneity with which GSH is distributed in the different types of lung cell makes it difficult, however, to compare individual GSH concentrations.

MDI conjugated to GSH is absorbed and is found in the blood as a protein adduct, particularly in the form of albumin and haemoglobin adducts. These protein adducts are produced by direct transcarbamoylation, as was demonstrated with toluene diisocyanate (TDI) (Day et al. 1997; Lange et al. 1999), and not, as formerly assumed, via the free amine.

Via hydrolysis, the corresponding amines or esters of carbamic acid can be produced from isocyanates which then react via a sequence of further reaction steps with still available isocyanate (and humidity) to form complex polyurea mixtures. This polymerized and usually precipitated material with a high-molecular weight is not bioavailable. It is eliminated via the gastrointestinal tract by mucociliary clearance (see Figure 2). This reaction is typical only for the behaviour of pure MDI in an aqueous medium. The reaction can be directed by dimethyl sulfoxide (DMSO), for example, towards the increased formation of the low-molecular amine (MDA) (solvolysis). Since also used as solvent DMSO has a decisive influence on the resulting products, the results of studies in which DMSO or similar vehicles were used are not suitable for the toxicological evaluation of MDI.

Mode of action in lung tumour formation

In two long-term studies with rats, locally irritating concentrations of MDI produced lung tumours at the site of deposition. Two principal mechanisms are conceivable for the tumour formation:

1. a genotoxic mechanism via the formation of MDA, which in animal experiments was found to be carcinogenic in the liver after oral administration
2. a non-genotoxic mechanism via the local irritating effects of MDI in the lungs.

ad 1.)

Whereas the formation of MDA cannot be completely excluded the available toxicological data yielded no evidence that MDA makes a relevant contribution to the toxicity of MDI after inhalationNo free MDA was determined in the urine or blood in either humans (Sennbro et al. 2003) or animals (Gledhill et al. 2005) after exposure to MDI. Merely the formation of MDA-GSH conjugates and acetylated MDA-GSH conjugates was detected (see Section 3.2).

- In agreement with this, no binding to DNA was determined in the lungs, the target organ for MDI toxicity, or in other organs after long-term inhalation of MDI (Vock et al. 1996). In contrast, MDA produced DNA adducts in the liver after oral administration (EU 2001). With MDI, only a very low level of DNA binding was found in the olfactory epithelium (Vock et al. 1996); this was possibly a pseudo-effect resulting from the marked binding to protein. The toxicological relevance of this observation is questionable, as MDI does not produce degeneration in the olfactory epithelium until high concentrations are reached.
- In animal studies, MDA produced liver damage as well as tumours of the liver and thyroid gland after oral administration (EU 2001). In micronucleus tests, MDA had a chromosome-damaging effect (EU 2001). In contrast, only effects in the respiratory tract were described in a large number of inhalation studies with MDI, which yielded negative results in a valid micronucleus test. Consequently there is no evidence of the biologically relevant formation of MDA after exposure to MDI.

ad 2.)

On the basis of all available data, a mechanism via the local irritating effects of MDI in the lungs is considered relevant (AGS 2000; EU 2005; Gledhill et al. 2005; Kilgour et al. 2002; Pauluhn et al. 1999 a; Reuzel et al. 1994 b; see also Figure 2):

- The initial event results from the interaction of MDI or PMDI with the surfactant system of the lungs. In this case, MDI apparently reacts with nucleophilic scavenger molecules with subsequent surfactant destabilization. Complexes made up of completely reacted MDI and precipitated surfactant are phagocytized by alveolar macrophages or eliminated via mucociliary clearance from the site of deposition (Pauluhn 2000, 2002 b). The initial event is accompanied by alveolar protein exudation; increased concentrations of total protein serve as a measurement of acute

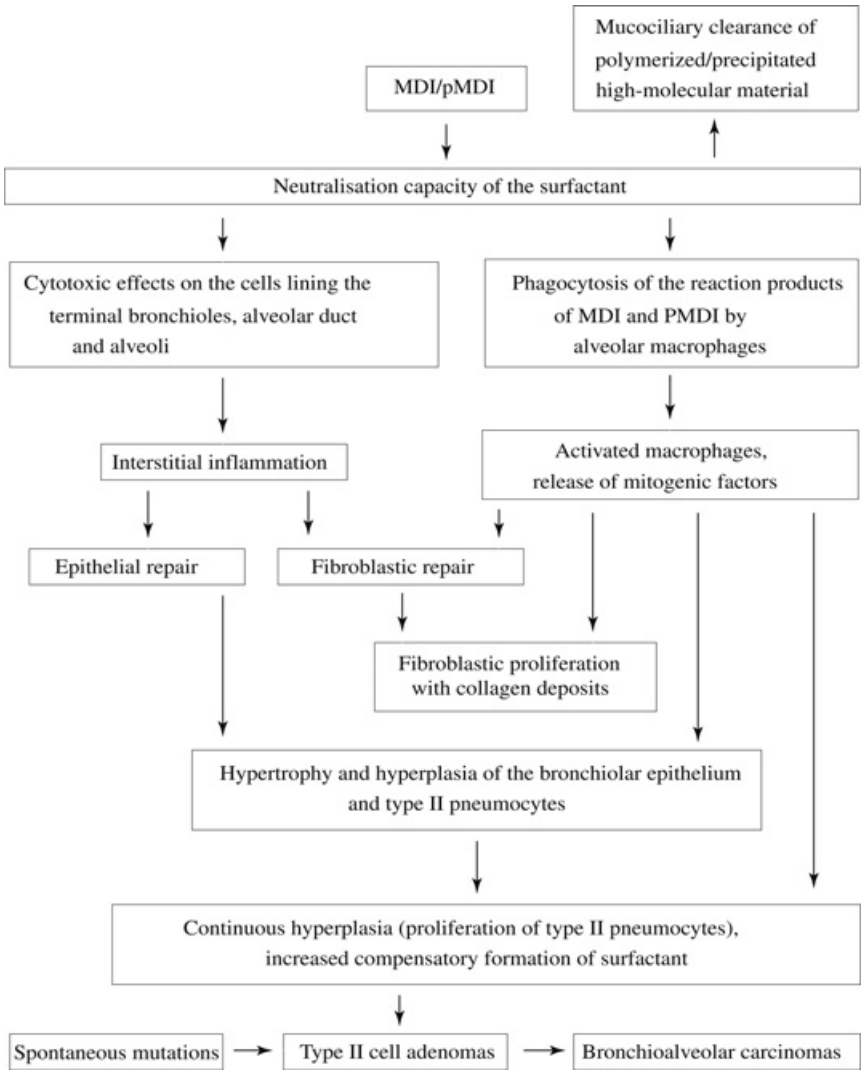

Figure 2 Diagram of the mechanism of action of MDI and PMDI (according to Reuzel et al. 1994 b, modified)

alveolar irritation (Kilgour et al. 2002; Pauluhn 2000; Pauluhn et al. 1999 a). The available findings thus support the hypothesis that lung tumours are caused by chronic regenerative cell proliferation and not by genotoxic initiation and promotion. It is furthermore noteworthy that the acute effects of irritation and the cor-

responding long-term end points correspond; there are no indications of cumulative or superadditive effects.

- Fibrotic effects can be produced as secondary effects to the chronic plasma exudation in the alveolar region. Similar findings have been described also for substances with a similar action profile (alveolar irritation) (Pauluhn et al. 2007).
- Alternatively, hypertrophy and hyperplasia of type II pneumocytes can be produced by the reactions of MDI with the surfactant or as a result of the cytotoxic effects of type I pneumocytes. This aetiopathology would not be in conflict with the above hypothesis.
- The increased regenerative proliferation of type II cells, produced either after damage as a result of irritation caused by type I pneumocytes or after a chronic increase in the surfactant synthesizing capacity (compensation of the surfactant that reacted with MDI), is thus considered to be the cause of preneoplastic changes in the rat. These findings represent a known chronic reaction of the rat lung to irritating substances such as quartz dust (Friemann et al. 1999).

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

Inhalation

In rats exposed by inhalation to ^{14}C-MDI for 15 minutes, a total of 70% of the absorbed quantity was eliminated after four days (see documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997). Immediately after the end of the inhalation exposure to ^{14}C-**MDI** concentrations of 0.06 or 0.4 mg/m^3, radioactivity was found in rats in the airways, in the gastrointestinal tract and in the circulatory system (see documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997; Brown et al. 1994; International Isocyanate Institute Inc. 1998).

In a study with ^{14}C-**MDI** carried out according to OECD Test Guideline 417, 28 male Wistar rats (Alpk:APfSD) were exposed once head only to concentrations of 2 mg/m^3 (nominal concentration, analysed concentration 3.67 mg/m^3, aerosol, MMD 1.39 µm) for 6 hours. The animals were killed directly after exposure or 8, 24 and 168 hours after the end of exposure. In another group, a bile duct cannulation was inserted and urine, bile and faeces were collected during the exposure and up to 48 hours after the end of exposure. The highest amounts of radioactivity were found in the respiratory tract within the first 8 hours after exposure and in the excretory organs thereafter. After 168 hours only traces were still detectable. Within 48 hours, 12% was found in the urine, 14% in the bile and 34% in the faeces of the animals with bile duct cannulation. No radioactivity was found in the CO_2 of

the expired air. Directly after the end of exposure, 32% of the radioactivity was found in the gastrointestinal tract and 34% of the radioactivity was eliminated with the faeces in the animals with bile duct cannulation; it is therefore assumed that a large proportion of the substance was absorbed orally and not by inhalation (for example during grooming). Most of the radioactivity had been eliminated within 168 hours; about 5% of the radioactivity was eliminated with the urine and about 79% with the faeces (Gledhill et al. 2005).

In a study with **PMDI** comparing the sensitivity of exposure and effect markers, female Wistar rats were exposed nose only to concentrations of 0 or 12.9 mg/m^3 for 6 hours a day on 5 days a week over 3 consecutive weeks. Interim sacrifices were performed on days 1, 4 and 11 and during the recovery period on days 18 (1 day after the end of exposure), 21, 28 and 35. As exposure markers, mainly MDA and acetylated MDA were determined after total hydrolysis in haemoglobin, plasma proteins, urine and bronchoalveolar lavage (supernatant and cells). In addition, also oligomeric MDA was determined. Changes in the bronchoalveolar lavage (see Section 5.2) were used as effect markers for lung toxicity. Both the concentrations of MDA and acetylated MDA, hydrolysed from the haemoglobin and protein adducts, and the parameters in the BAL (mean cell volume, total cell count, phospholipid and protein concentrations) were increased during the exposure and decreased rapidly after the end of the exposure. Markedly lower amounts of oligomeric MDI and PMDI were recovered than of MDI (1:0.7 and 1:< 0.1); these substances are eliminated by clearance via the airways or via phagocytosis (Pauluhn 2002 a).

Pregnant Wistar rats were exposed to **MDI** concentrations of 20 mg/m^3 for 6 hours (aerosol, whole body exposure) on gestation day 19. Immediately after exposure, blood from the dams, amniotic fluid, foetuses and the placenta were collected. MDA was found after acid hydrolysis; no differentiation was made between MDI and MDA. The highest concentrations were found in the blood of the dams (12.5 ng/g), followed by the placenta (8.3 ng/g), foetuses (5.3 ng/g) and amniotic fluid (1.7 ng/g) (UBA 1996).

Dermal exposure

After non-occlusive dermal application of ^{14}C-**MDI** in acetone in doses of 2.6 to 6.9 mg per rat (10.9–29.8 mg/kg body weight), 10% to 12% of the radioactivity was still detectable in the epidermis after 24 and 48 hours. Distribution in the tissues was more or less even, though somewhat higher in the tongue, indicating at least partial oral uptake. Within 24 hours, 16% to 22% of the applied radioactivity was eliminated with the faeces, and 6% to 15% over the following 24 hours. Elimination with the urine was markedly lower at 0.6% to 0.8% within the first 24 hours, and 0.2% to 0.3% over the following 24 hours. Only about 50% was recovered (Vock and Lutz 1997). Under the conditions of this study, oral uptake of MDI via grooming of the pelt is to be assumed.

In another study with ^{14}C-**MDI**, 0.4 or 4 mg/cm^2 in acetone was applied dermally (under occlusive conditions) for 8 hours or 0.4 mg/animal in corn oil intradermally to four male Wistar rats. Under these test conditions, a maximum 0.9% of the radioactivity was absorbed after dermal application; most of the radioactivity was detectable on the skin (0.4 mg/cm^2: up to 55.6%; 4 mg/cm^2: up to 32.2%) and in the bandage material (0.4 mg/cm^2: up to 50%; 4 mg/cm^2: up to 69.1%). After intradermal administration, 26% of the radioactivity was absorbed (EU 2005).

In an in vitro study of the dermal absorption of ^{14}C-**MDI**, 0.04 or 4 mg/cm^2 in acetone was applied under non-occlusive conditions to the skin of guinea pigs, rats and humans. In all species, most of the radioactivity (58%–91%) remained on or in the skin. No radioactivity was absorbed by human skin during the 54-hour exposure period. The skin of rats and guinea pigs absorbed very small quantities of about 0.14% of the applied dose (International Isocyanate Institute Inc 1997).

In a study with monkeys that examined the decontamination after skin contact, it was shown that decontamination using polypropylene glycol, polyglycol-based cleansers or with corn oil is superior to washing with soap and water (Wester et al. 1999). In a study in which **PMDI** was applied occlusively to the skin of rats in doses of 100 mg/kg body weight for 8 hours, the MDA was determined after total hydrolysis of the blood and urine, and its bioavailability was found to be < 0.01% (Pauluhn and Lewalter 2002).

A comparative review of these studies yielded no conclusive evidence that MDI is absorbed through the skin in toxicologically relevant amounts. In the studies considered, it is not clear whether the MDI was recovered systemically in the form of a GSH adduct or as a sequel of the catabolism of conjugated skin proteins. In addition, the influence of aqueous decontamination systems (termination of skin exposure by decontamination) on the investigated analytes was not investigated sufficiently. The data of Pauluhn and Lewalter (2002) show that the purported "bioavailability" of hydrolysis products of MDI is increased by skin decontamination.

3.2 Metabolism

MDI forms GSH mono or bis-conjugates. Where there is an excess of GSH, as in the respiratory tract, the GSH bis-conjugate is predominant (Pauluhn et al. 2006; see Figure 3). In vitro investigations showed that the MDI GSH bis-conjugate is relatively unstable with a half-life of 59 minutes, whereas the MDA-GSH mono-conjugate is much more stable under the same conditions (25°C, pH 7.4) with a half-life of 470 minutes (Reisser et al. 2002). As the mono-conjugate has a longer half-life in vitro, conjugated MDI is probably present in vivo in the form of the mono-conjugate. This is the basis for the formation of the acetylated metabolites identified as listed below; these were demonstrated in the urine, faeces or bile of rats after 6-hour exposure to ^{14}C-**MDI** concentrations of 2 mg/m^3 (Gledhill et al. 2005; see Figure 3):

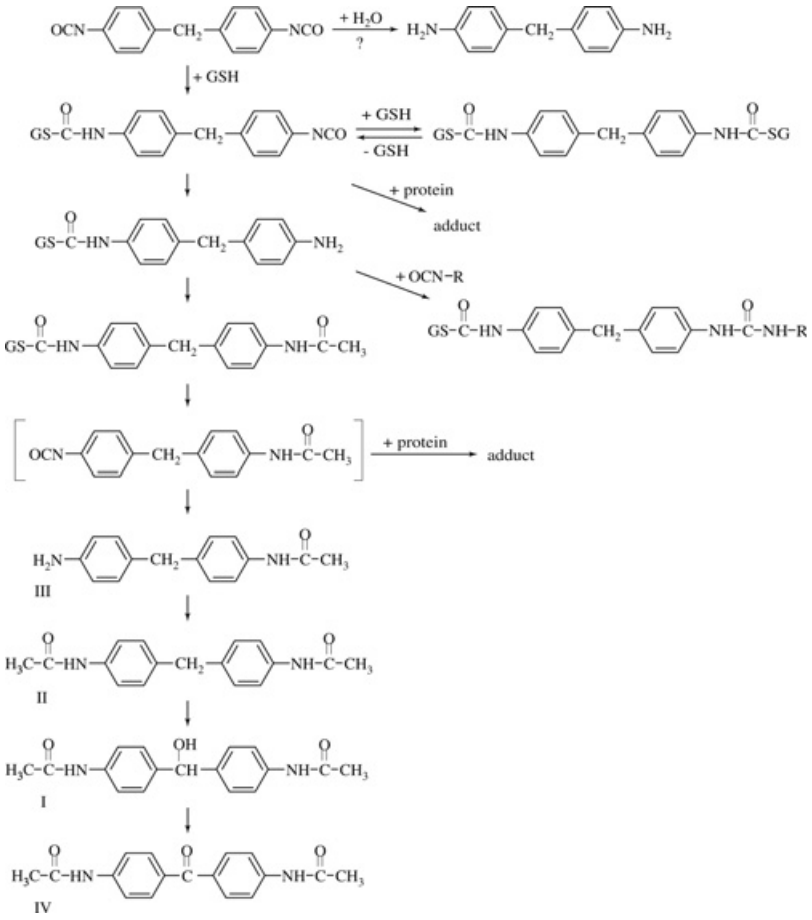

Figure 3 Metabolism of MDI (metabolites I-IV e)

- Metabolite I: *N,N′*-diacetyl-4,4′-diaminobenzhydrol (1% or 6% in the urine of intact animals or animals with bile duct cannulation, 1% in the bile)
- Metabolite II: *N,N′*-diacetyl-4,4′-diaminophenyl methane (0.5% or 4% in the urine of intact animals or animals with bile duct cannulation, 4% in the bile)
- Metabolite III: *N*-acetyl-4,4′-diaminophenyl methane (0.3% in the urine)
- Metabolite IV: *N,N′*-diacetyl-4,4′-diaminobenzophenone (0.4% in the urine)
- Metabolite V: "minor metabolite" not characterized (0.2% in the urine)
- Peak VI: polyurea oligomers of different molecular weights (not detected in the urine, 9% in the bile, 24% in the faeces).

Protein = Protein
Addukt = Adduct

Free MDA was not detected in either the urine, faeces or bile (Gledhill et al. 2005).

Furthermore, on dialysis of the plasma no MDA was found in the dialysate or urine of workers exposed to MDI. In addition, it was shown that MDI metabolites in the plasma are found only when bound to protein. Also in the urine, no free MDA was detectable; all metabolites were conjugated (Sennbro et al. 2003).

On the basis of these data, the metabolic pathway shown in Figure 3 is assumed.

3.3 Binding to macromolecules/biological monitoring

Protein adducts

Around 95% of the total MDI adducts in human plasma are albumin adducts (Johannesson et al. 2004; Sennbro et al. 2003). A 70-kDa protein adduct was found as the main product in the blood of rats after 4-hour inhalation of ^{14}C-**MDI** (0.06 and 0.4 mg/m^3) (Brown et al. 1994).

In rats, after dermal administration of 2.6 to 6.9 mg ^{14}C-**MDI** per animal (10.9–29.8 mg/kg body weight), the extent of covalent binding in the nuclear protein of the epidermis was very pronounced with up to 10^6 dpm/mg, but 10 000 times lower in the liver, kidneys and lungs (Vock and Lutz 1997).

Haemoglobin adducts

After exposure of rats to **PMDI** concentrations of a maximum 12.9 mg/m^3 for 6 hours or 14 days, MDA was detected after the hydrolysis of haemoglobin, but acetylated MDA was found in 2 to 5-fold higher quantities (Pauluhn 2002 a; Pauluhn and Leng 2003). Corresponding haemoglobin adducts were determined also after treatment with MDA (Kautiainen et al. 1998). In Wistar rats exposed to **MDI** for 3 months in concentrations of up to 2 mg/m^3 for 17 hours a day on 5 days a week, a dose-dependent, specific haemoglobin adduct (MDA-Val-Hyd) was identified after the acid hydrolysis of globin. This was found in concentrations about 12 times higher than the sum of MDA and acetylated MDA after mild alkaline hydrolysis of haemoglobin (Sabbioni et al. 2000).

In a study with 3 and 12 months exposure to maximum **MDI** concentrations of 2.06 mg/m^3, no changes in the amounts of haemoglobin adducts, hydrolysed MDA and acetylated MDA in the urine were found, which suggests a steady state between adduct formation and elimination (Sepai et al. 1995 b). Only slight differences regarding the haemoglobin adducts and the metabolites in the urine were observed after single whole-body or nose-only exposures to **MDI** concentrations of 20 mg/

m^3 for 6 hours (Sepai et al. 1995 b). MDA and acetylated MDA were also found after neutral or alkaline extraction of haemoglobin following oral or intraperitoneal doses of **MDI** of 0.5 mmol/kg body weight. After intraperitoneal injection, the haemoglobin binding index (HBI) was 0.025 for MDA and 0.038 for acetylated MDA; after oral administration, an HBI of 0.011 was obtained for MDA and of 0.029 for acetylated MDA (Sabbioni and Schütze 1998).

In a study, male Wistar rats were exposed to **PMDI** either by inhalation of 3.7 or 12.7 mg/m^3 ($C \times t = 1332$ and 4572 mg/m^3 and hour) for 6 hours, or **PMDI** was applied dermally in doses of 100 mg/kg body weight. MDA was used for comparison. Haemoglobin adducts were determined after acid hydrolysis as MDA or oligomeric MDA. The results differed as regards the two routes of absorption and the exposure to PMDI and MDA. The proportion of acetylated adducts was markedly higher after the exposure to PMDI than after exposure to MDA, and absorption through the skin was many times greater for MDA than PMDI. Unlike with MDA, there were clear differences between the routes of absorption with PMDI, which is probably attributable to GSH conjugation in the respiratory tract. No evidence was found of the spontaneous hydrolysis of MDI to MDA (Pauluhn and Lewalter 2002).

Biological monitoring

Exposure to MDI can be demonstrated by determining MDA in hydrolysed urine or hydrolysed protein (plasma, erythrocytes) (Dalene et al. 1996; Kääriä et al. 2001; Schütze et al. 1995; Sennbro et al. 2006; Skarping et al. 1994, 1995, 1996; Pauluhn et al. 2006). Hydrolysis is necessary to dissolve the binding of MDI to biological materials, for example protein. This method therefore does not allow any quantitative statements to be made about the extent of the possible intermediary formation of free MDA or other sequel products from MDI in the organism (Pauluhn et al. 2006). In a controlled animal study, the correlation between the MDI concentration in the air and biomarkers in the urine as markers of recent exposure is high (Pauluhn and Leng 2003). MDA is determined after hydrolysis in the urine also in biomonitoring studies in workers exposed to MDI. In 2006, a BLW ("**B**iologischer **L**eit**w**ert" = see List of MAK and BAT Values, section XIV) of 10 µg MDA per litre urine was established for MDI (Drexler and Greim 2007).

Haemoglobin adducts and (human) serum-albumin adducts are cumulative biomarkers of individual isocyanate exposure, whereby haemoglobin adducts are suitable as markers of previous exposure and serum-albumin adducts represent markers of current exposure (Lewalter and Steinmann-Steiner-Haldenstaett 1994; Sepai et al. 1995 a). Systematic studies have shown that the yield of biomarkers in the relevant matrix depends to a great extent on the method of hydrolysis used, the relationship between exposure and sampling times and, particularly, on the exposure route (dermal or by inhalation). The methods used today are not isocyanate-specific; this means extracorporeal contamination in the form of absorbable precur-

sor or breakdown products of MDI can produce false-positive results (Pauluhn 2002 a; Pauluhn and Leng 2003; Pauluhn and Lewalter 2002).

4 Effects in Humans

4.1 Single exposures

It is known from earlier reports that short-term exposure to **PMDI** can cause irritation of the eyes and respiratory tract (see documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997).

4.2 Repeated exposure

The impairment of lung function resulting from long-term exposure to **MDI** and **PMDI** has been investigated in a number of studies; the usefulness of some of these studies is limited by simultaneous exposure to toluene diisocyanate and inaccurate exposure data (see documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997; Petsonk et al. 2000). In these studies, a deterioration in lung function was observed at **PMDI** concentrations as low as 87 ppb (0.9 mg/m^3). Where a maximum concentration of 20 ppb (0.2 mg/m^3) was generally observed, no significant changes in lung spirometry were found, although airway symptoms occurred in a number of persons. After exposure to PMDI concentrations of below 10 ppb (0.1 mg/m^3) the frequency of respiratory complaints was not significantly increased (see documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997). More recent studies are not available.

4.3 Local effects on skin and mucous membranes

As mentioned above, **PMDI** can cause irritation of the eyes and respiratory tract.

4.4 Allergenic effects

4.4.1 Airways

MDI can cause specific or non-specific hyperreactivity of the airways.

Non-specific hyperreactivity

Isocyanate asthma is usually accompanied by non-specific bronchial hyperreactivity (see documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997), the severity of which can vary markedly, both interindividually and intraindividually. This applies both to the attack-free interval and the asthma attack. Correspondingly, a higher incidence of non-specific airway reactions has been reported also in workers exposed to MDI (Jang et al. 2000).

Specific hyperreactivity

As regards specific hyperreactivity, asthma (immediate-type, late-onset reaction or dual type) is markedly more frequent than exogenous allergic alveolitis (Baur 1996; Schreiber et al. 2006; Vandenplas et al. 1993 a, b).

Bronchial asthma is known to be a pathological condition induced by diisocyanates such as MDI (see supplement "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 2000; Carino et al. 1997; EU 2005; Perfetti et al. 2003). On adherence to a **PMDI** concentration of below 5 ppb (0.05 mg/m^3), the new development of asthma attacks or asthmatic complaints was not observed (see documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997).

In a provocation test, one of two workers with asthma and rhinitis reacted to **MDI** after exposure to 5 ppb (0.05 mg/m^3) for 4 minutes with an immediate-type reaction, the other after 10 ppb (0.1 mg/m^3) for 10 minutes with a late-onset reaction. No reaction was observed after 45-minute exposure to 5 ppb (0.05 mg/m^3) (Baur et al. 1996). In further provocation tests with 54 patients, six reacted to 5 ppb (0.05 mg/m^3), two to 10 ppb (0.1 mg/m^3) and eight to 20 ppb (0.2 mg/m^3), 38 did not react up to 20 ppb (0.2 mg/m^3) (Baur et al. 1994). In two publications, provocation tests were carried out with 3 and 5 ppb (0.03 and 0.05 mg/m^3) in 22 and 24 patients with suspected occupational isocyanate asthma. Two patients reacted to 3 ppb (0.03 mg/m^3), the other 22 patients were also exposed to 5 ppb (0.05 mg/m^3). Fourteen of the persons exposed to MDI developed bronchial hyperreactivity in the methacholine test, five persons developed asthma symptoms after exposure to MDI (Barbinova and Baur 2006; Baur and Barbinova 2005).

In a study with 243 workers in polyurethane processing with exposure to MDI (continuous determination), in which the threshold limit value of 5 ppb (0.05 mg/m^3) was continuously observed and occasional skin contact occurred, increased specific IgE and IgG values in the serum were found in two persons, in one case combined with a prick test reaction to MDI human serum albumin and cutaneous anaphylaxis. Occupational asthma was diagnosed in three persons. A relationship with the MDI exposure, which in the meantime was increased, was assumed, but not verified by a provocation test (Bernstein et al. 1993).

Exposure to **MDI** concentrations of ≥ 5 ppb (0.05 mg/m^3) is associated with the increased occurrence of occupational asthma (Tarlo et al. 1997). In some cases of occupational asthma caused by MDI, contact urticaria was described (Kanerva et al. 1999 b; Valks et al. 2003;). In a case report, asthma from contact with plaster casts containing MDI is described in a female nurse (Donelly et al. 2004).

In a small case–control study (7 cases), the cases of asthma associated with MDI more often had past exposure above 5 ppb (0.05 mg/m^3) (odds ratio (OR) 7.5; 3/7 compared with 1/11 of the controls) (Meredith et al. 2000).

The results of RAST (radioallergosorbent) tests to demonstrate specific IgE antibodies correlate only poorly with the clinical symptoms. Negative results are obtained in exposed healthy persons, whereas positive RAST results were described in some exposed persons with respiratory symptoms (Baur et al. 1994; Diller 1991). Although MDI-specific IgG correlated with the exposure, it is not an indicator of MDI-induced occupational asthma (Lushniak et al. 1998).

4.4.2 Skin

Toxic or allergic contact dermatitis from MDI has occasionally been described (Frick et al. 2003 a; Iorizzo et al. 2001; Kanerva et al. 1994, 2001; Mancuso et al. 1996; Militello et al. 2004; Schröder et al. 1999; Sertoli et al. 2004). In a study of 120 patients with building industry occupations, 105 of whom reported occupational dermatosis, none reacted in patch tests to 0.1% MDI (Geier and Schnuch 1998). In a patient with contact dermatitis from a plastic watch strap, the patch test with MDI (1% and 2% in methyl ketone) yielded negative results (Alomar 1986). Of 22 patients in whom occupational contact dermatitis from isocyanates or polyurethane was diagnosed in a Swedish hospital between 1978 and 2001, six reacted to MDI (0.1% or 1% in petrolatum) (Goossens et al. 2002). In a study comprising 17 patients with occupational contact dermatitis, none reacted (2% MDI in petrolatum) (Frick et al. 2003 b). Of 179 patients with suspected occupational contact dermatitis who were investigated between 1991 and 1996 using the patch test (2% MDI in petrolatum), none reacted (Kanerva et al. 1999 a). One publication reports that MDI concentrations in commercial patch test preparations are often too low, so that false-negative results in the patch tests are conceivable (Frick et al. 2004).

4.5 Reproductive and developmental toxicity

There have been no more recent studies since the documentation from 1992 (documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997).

4.6 Genotoxicity

One abstract reports an increase in chromosomal aberrations in persons exposed during the production of polyurethane (Szekely and Gundy 1999). However, as no information was given in particular about the levels and duration of exposure, but also about other methodological details, this study cannot be included in the evaluation.

In addition, in the EU Risk Assessment, abstracts are cited which reported a slight increase in sister chromatid exchange (SCE) in peripheral lymphocytes or micronuclei in buccal mucosa cells in workers exposed to MDI. Here too, however, evaluation is not possible as a result of inadequate documentation. In a publication about female workers in two shoe factories with exposure to a complex mixture of various solvents including MDI, an increase in micronuclei in the lymphocytes was described, but no increase in SCE (Pitarque et al. 2002). In a second publication by the same research group, no DNA damage in monocytes was detected using the comet assay (Pitarque et al. 1999).

In an investigation in a worker, DNA double strand breaks and MDI crosslinks in white blood cells were observed two hours after exposure to MDI concentrations of 5 to 20 ppb (0.05–0.2 mg/m^3) (Marczynski et al. 1992). This investigation was likewise strongly criticized on account of methodological shortcomings such as the lack of tests for isolation artefacts, the lack of suitable controls or standards and misinterpretation of the results (EU 2005). Two other insufficiently documented studies by the same research group report DNA fragmentation in the white blood cells of workers after short-term exposure to MDI concentrations of 2 ppb (0.02 mg/m^3) for 15 minutes, 5 ppb (0.05 mg/m^3) for 60 minutes and 10 ppb (0.1 mg/m^3) for 5 minutes (Marczynski et al. 1994 a, b). In another study by this research group, 10 patients with workplace-related dyspnoea, who had presented themselves for a presumed occupational medical examination, were exposed to **MDI** in exposure chambers. The exposure consisted of 5 ppb (0.05 mg/m^3) and then 10 ppb (0.1 mg/m^3) for 30 minutes followed by a 90-minute interval, then 20 ppb (0.2 mg/m^3) for 30 minutes followed by a second 90-minute interval, and finally 30 ppb (0.3 mg/m^3) for a further 30 minutes. Blood samples were taken prior to exposure as well as 30 minutes and 19 hours after exposure. The white blood cells (no other details) were investigated using gel electrophoresis for low-molecular weight (LMW) DNA fragmentation before and after exposure. Eleven untreated persons served as controls. There was no difference in DNA fragmentation patterns prior to exposure, with one exception. In one female patient who reacted in the challenge to MDI there was already an increase in DNA fragments before exposure. After exposure, changes in the DNA fragmentation pattern such as observed also after exposing cells to H_2O_2 were found in 4 of 10 exposed persons. This was regarded as evidence of a change in the intracellular redox steady-state. According to the authors, there was no relationship between DNA fragmentation and potential confounders such as age, smoking status and medication. There were, however, differences between the exposed persons and the controls regarding these factors

(Marczynski et al. 2003). As a result of methodological shortcomings, also the results of this study cannot be included in the evaluation. For example, the whole blood samples were frozen down to –20°C without relevant protection and thawed at room temperature, both of which can produce cellular lysis. In addition, the gels for electrophoresis were overcharged, and clearly degraded DNA and RNA fragments could be seen—evidence of cellular lysis. Also, the cell counts used and the DNA quantities were not defined.

For a comet assay, 25 patients with workplace-related dyspnoea were exposed to **MDI** concentrations of 0.05, 0.1, 0.2 and 0.3 mg/m^3 in chambers for 30 minutes using the same method as described by Marczynski et al. (2003). No increase in DNA strand breaks was observed in the lymphocytes of the exposed patients in a valid comet assay conducted in accordance with present-day requirements (Marczynski et al. 2005).

4.7 Carcinogenicity

4.7.1 Case reports

Although a varnisher with occupational bronchopulmonary disease who had been exposed to MDI and TDI for 15 years developed a lung carcinoma, no further data are available to substantiate a causal relation between the exposure to MDI and the tumour (EU 2005).

4.7.2 Epidemiological studies

The incidence of tumours and mortality were determined in a cohort study comprising 4154 workers employed in 9 different polyurethane foam plants in Sweden for at least one year up to 1987. Exposure to TDI or MDI was determined qualitatively in retrospect. Exposure to MDI occurred in 8 of the 9 factories. Sporadic random samples from 1965 onwards yielded average analysed MDI concentrations of < 0.01 mg/m^3. Maximum MDI concentrations of up to 0.35 mg/m^3 were found. The corresponding TDI concentrations were < 0.1 mg/m^3, with peak values of 3 mg/m^3. In addition to the isocyanates, the workers were exposed also to other chemicals in non-quantified amounts such as solvents, foaming agents, lubricants and amine accelerators. Information regarding the vital status and cause of death in the cohorts was obtained from Statistics Sweden. Information about tumours was obtained from the National Swedish Tumour Register. The deaths were compared with the deaths in the population of Sweden to compensate for differences in age distribution and the duration of subsequent monitoring. There was a statistically significant reduction in mortality in the cohorts [130 cases; standardized mortality ratio (SMR): 0.78; 95% confidence interval (CI): 0.66–0.93]. This applied also for all malignant neoplasms, although the reduction was not statistically significant (SMR:

0.77; 95% CI: 0.53–1.09). A lower tumour incidence was likewise found compared with that in the population [72 cases; standardized incidence ratio (SIR): 0.81; 95% CI: 0.63–1.02]. When individual localizations were considered, there were increased incidences for rectal cancer (6 cases; SIR: 1.66; 95% CI: 0.61–3.61) and for non-Hodgkin's lymphomas (4 cases; SIR: 1.53; 95% CI: 0.42–3.91). The increase was not statistically significant in either case (Hagmar et al. 1993 a). To obtain more detailed information about exposure, a nested case–control study was carried out. The workplace description was drawn up by the occupational safety personnel responsible, thus allowing a more accurate exposure estimate to be made (extent of exposure, chemicals). The non-significantly increased incidences could not be confirmed in this nested case–control study. An association was found between high isocyanate exposures and the formation of prostate cancer (OR: 2.66; 90% CI: 0.39–18.1), which was not statistically significant (Hagmar et al. 1993 b). The usefulness of this cohort is limited as a result of the short observation period and the resultant low mortality and low number of tumours.

A further follow-up study of this cohort, which comprised the period between 1959 and 1998, was published in 2004 and included a total of 337 deaths. The mortality deficit (SMR: 0.84; 95% CI: 0.75–0.93) compared with Swedish mortality statistics was hereby confirmed. The incidence of tumours was 209 and was lower than expected (SIR: 0.87; 95% CI: 0.75–0.99). Mortality was not increased for either the tumours in total or for chronic lung diseases. The only unusual findings were an increase in the incidence of mortality from lung tumours in the women (10 or 12 cases; SMR: 3.52; SIR: 3.00) and an increase in malignant tumours in the brain and nervous system in men and women (SIR: 1.49; with a latency period of at least 20 years since the first exposure SIR: 2.45). It can be assumed, however, that lung tumours resulted in double citations for both incidence and mortality. The lung tumours in the women were investigated further in a nested case–control study. No association between the lung tumours and exposure to polyurethane dust was found (OR: 0.8) (Mikoczy et al. 2004). The usefulness of the study is limited by the absence of information regarding smoker status and the low total number of tumours. In the authors' opinion the study provides evidence of an increase in lung tumours in women in polyurethane foam production, the cause of which is unclear.

The results of an English study are also available. A cohort consisting of 8288 male and female workers from 11 different British polyurethane foam factories, 7 of which used MDI, was compared with the population of England and Wales with regard to causes of death. The persons were employed for at least 6 months in the period between 01.01.1958 and 31.12.1979. The isocyanate concentrations were in the range of > 4 ppb (0.016 mg/m^3), 1.5–4 ppb (0.006–0.016 mg/m^3) and < 1.5 ppb (< 0.006 mg/m^3). There was no information given about the amount of TDI and MDI or details of exposure to other chemicals. A total of 816 workers (SMR: 0.97) died during the subsequent observation period, 221 persons from a malignant neoplasm (SMR: 0.88). The tumour incidence was, with 277 cases, lower than expected (294.3 cases; SIR: 0.94). In women, significantly increased incidences were given for lung carcinomas (17 cases; SIR: 2.33; 95% CI: 1.36–3.73). The authors draw

attention to the higher prevalence of smoking among the female workers which, together with other non-occupational factors, may explain the increased SMR for lung cancer. In the whole cohort, an increased SMR for pancreatic cancer (tumour type not stated) was observed (14 cases; SMR: 136). A sex-specific evaluation revealed significantly increased mortality in women from pancreatic cancer (6 cases; SMR: 271) and the expected number of deaths in men (Sorahan and Pope 1993). As the maximum study period of 22 years was too short, a further study based on the same cohort was carried out covering the period from 1958 to 1998. In this publication, exposure to isocyanates was stated to be mainly in the form of TDI, but not further described. 1662 deaths were observed compared with an expected value of 1547.9 (SMR: 1.07). Compared with the general population in England and Wales, mortality from lung cancer was increased in the women, but not in the men (SMR: 181; 95% CI: 1.26–251). In the evaluation of isocyanate exposure, no direct correlation between isocyanate exposure and lung cancer or non-malignant diseases of the respiratory tract could be found. Hence, the authors do not assume there to be an association between lung cancer mortality and exposure to isocyanates (Sorahan and Nichols 2002). This interpretation by the authors was, however, questioned in a reader's letter (Mirer 2003). In their reply, Sorahan and Nichols stated that none of the women with lung cancer were actually occupationally exposed to isocyanates (Sorahan and Nichols 2003). In a further publication, details of the smoking habits of some persons of the cohort were published (Clark et al. 2003). Here, the lung function in a group of 251 workers in flexible foam production was recorded, 30 of whom were women with exposure to TDI. This was a subcohort of the population in the Sorahan study which was investigated over a period of 17 years and for whom data about smoking habits were collected. These data show that the prevalence of smokers in the exposed group (n = 175) was higher (54%) than in the low-exposure group (n = 50; 40%). Although the number of women is small, the proportion of female smokers would seem to be increased.

In a case–control study, the relationship between 293 substances at the workplace and various types of tumour was investigated. The substances included isocyanates, TDI being the most frequent. Exposure to isocyanates was assumed in car mechanics and foundry workers. Overall, only 0.8% of the investigated workers were exposed to isocyanates. The exposure to isocyanates correlated with a series of other chemicals, for example polycyclic aromatic hydrocarbons, inorganic pigments, organic paints and pigments, and chromium(VI) compounds. Adjustments for known non-occupational confounders were carried out in the statistical evaluation. In the case of lung cancer, the adjustments were for age, family income, smoking habits, ethnic origin and alcohol consumption. No adjustments were carried out, however, for the co-exposures. No increased ORs were found for the investigated tumour locations or types (oesophagus, stomach, large intestine, rectum, pancreas, prostate, bladder, kidneys, melanoma, lymphoma), with one exception. In the Francocanadian subgroup (the largest ethnic group in the region), but not for the total population, an OR of 2.2 (90% CI: 0.9–5.3) was determined for lung cancer on the basis of ten cases (all exposure levels). No association was found between lung

cancer and high isocyanate exposure (Siemiatycki 1991). In view of the large number of chemicals, tumour types and potential associations investigated, this study must be regarded as explorative. In addition, the analyses related to lung cancer were not adjusted for co-exposure to potential lung carcinogens.

Summary

Studies with exposure to only or mainly **MDI** or **PMDI** are not available. In the studies described (Hagmar et al. 1993 a, b; Mikoczy et al. 2004; Sorahan and Pope 1993; Sorahan and Nichols 2002), both mortality and the incidence of lung tumours were increased in two cohorts of exposed women in polyurethane foam production (Mikoczy et al. 2004; Sorahan and Pope 1993). However, further studies were not able to confirm a relationship between exposure to diisocyanates and lung tumours (Mikoczy et al. 2004; Sorahan and Nichols 2002). On account of the exposure to a mixture of substances, unspecified exposure concentrations for MDI or the absence of exposure to MDI, it is not possible to draw any conclusions regarding the potential carcinogenic effects of MDI in humans.

5 Animal Experiments and in vitro Studies

The main effects of **MDI** and **PMDI** are the effects on the respiratory tract.

5.1 Acute toxicity

MDI

The inhalation of **MDI** concentrations of 2240 mg/m^3 (aerosol, MMAD 4.9 µm, about 80% < 10 µm) for one hour resulted in the death of 1 of 10 rats. Clinical symptoms were, for example, difficult and irregular breathing, nasal discharge, red incrustations in the nasal region, unkempt fur and hypothermia (International Isocyanate Institute Inc 2003).

In rats exposed to radioactively labelled **MDI** for 4 hours, the radioactivity after concentrations of 0.05 mg/m^3 was associated with the epithelium of the airways. At 0.4 mg/m^3 the first signs of inflammation were reported, but without any details as to what parameters were changed. Damage to the epithelium was observed at 6 mg/m^3 (International Isocyanate Institute Inc 1998; see Table 1).

Table 1 Studies with rats with single inhalation exposures to MDI and PMDI

Species, strain, number of animals per group	Exposure, investigation	Findings	References
MDI			
rat, F344, 4 ♂ per group	**4 hours**, ^{14}C-MDI: 0.05, 0.4 mg/m^3; MDI: 6 mg/m^3, aerosol, MMAD 1.18 µm, investigation at end of exposure	**0.05 mg/m^3 and above**: radioactivity associated with the epithelium of the airways **0.4 mg/m^3 and above**: minimal evidence of inflammation (no other details) **6 mg/m^3**: epithelial damage	International Isocyanate Institute Inc. (1998)
PMDI			
rat, Wistar, 6 ♂ per group	C × t = 1200 mg/m^3 and min 6 hr × 3.4 mg/m^3, 3 hr × 6.2 mg/m^3, 1.5 hr × 12.7 mg/m^3, 45 min × 25.1 mg/m^3, 23 min × 58.1 mg/m^3	**0.5 mg/m^3**: estimated NOEC **3.4 mg/m^3 and above**: protein in the BAL ↑	Pauluhn 2002 b
rat, Wistar, 6 ♀ per group	**6 hours**, 0, 0.7, 2.4, 8 or 20 mg/m^3, aerosol, MMAD 1.5 µm, investigation after 0 or 3 hours or after 1, 3 or 7 days	**0.7 mg/m^3**: protein in the BAL ↑ (shortly after exposure, reversible within 1 day) **2.4 mg/m^3**: phospholipids, protein and ACE in the BAL ↑ **8.3 mg/m^3**: cell count ↑, γ-GT ↑, GSH in the BAL and in the lung tissue ↑ **20 mg/m^3**: relative lung weights ↑	Pauluhn 2000
rat, Wistar, 6 ♂ per group	**6 hours**, 0, 0.67, 2.4, 8.1, 20 mg/m^3, aerosol, MMAD 1.4–1.5 µm, investigation after 3 hours, 1, 3 or 7 days	**0.67 mg/m^3**: protein in the BAL ↑ (shortly after exposure, reversible within 1 day) **1.4 mg/m^3**: NOAEC for protein increase in the BAL **8.1 mg/m^3**: protein in the BAL ↑ **20 mg/m^3**: absolute lung weights ↑ (not significantly)	Pauluhn 2004 a
rat, Wistar or Brown Norway, 6–8 ♂ per group	**6 hours**, 0, 0.25, 2.5, 8.3, 19.5 mg/m^3, aerosol, MMAD 1.4–1.6 µm, investigation after 1 day (BAL) or after 1, 3 or 7 days (Penh)	**2.5 mg/m^3**: no effects on protein in the BAL (1 day after end of exposure) **8.3 mg/m^3**: clinical symptoms (laboured breathing), effects on functional respiratory parameters (Penh), protein in the BAL ↑	Pauluhn 2004 b

Table 1 (Continued)

Species, strain, number of animals per group	Exposure, investigation	Findings	References
		19.5 mg/m³: laboured breathing, nasal discharge, red incrustation of nostrils, transient body weight loss, relative lung weights ↑	
rat, Wistar, 4–6 ♂ per group	**2.5 hours**, 0, 2.4, 6.7, 15.8, 38.7 mg/m³, MMAD 1.4–1.6 µm	**2.4 mg/m³ and above**: first signs of lung irritation **38.7 mg/m³**: respiratory minute volume markedly ↓	Pauluhn and Leng 2003 Pauluhn et al. 1999 a
rat, Alpk:ApfSD, 5 ♀ per group	**6 hours**, 0, 10, 30, 100 mg/m³, nose-only, MMAD 0.96–1.25 µm, investigation after 1, 3, 10 or 30 days	**10 mg/m³ and above**: pneumonitis, total protein, total cell count, alveolar macrophages, leukocytes and lymphocytes in the BAL ↑, BrdU incorporation in terminal bronchioles ↑ **30 mg/m³ and above**: clinical symptoms (abnormal respiratory noises, breathing rate ↓, breathing depth ↑, discharge of mucous from the nose, lacrimation, reduced activity; all reversible), body weights ↓, lung weights ↑ (no details whether relative or absolute), alkaline phosphatase, *N*-acetylglucose aminidase and lactate dehydrogenase in the BAL ↑ **100 mg/m³**: hypoaesthesia, greater breathing depth	Kilgour et al. 2002

ACE: angiotensin converting enzyme; BAL: bronchoalveolar lavage; BrdU: bromodeoxyuridine; GSH: glutathione; NOAEC: no observed adverse effect concentration; NOEC: no observed effect concentration;
Penh: methacholine bronchoprovocation challenge with determination of the functional respiratory parameter "parameter enhanced pause" (Penh) by whole-body plethysmography;
γ–GT: γ–glutamyl transpeptidase;

PMDI

For **PMDI**, the LC_{50} after inhalation exposure for 4 hours was 490 mg/m³ (aerosol, 95.5% < 4.3 µm). Symptoms of intoxication were laboured breathing, breathing through the mouth, and a bloody discharge from the nose. Death occurred within 2 days (Reuzel et al. 1994 a).

In addition, a number of studies with rats with single inhalation exposures are available, in which the locally irritating effects of **PMDI** on the respiratory tract

were established—mostly by investigating the bronchoalveolar lavage. These studies are summarized in Table 1.

Bronchoalveolar lavage (BAL) is a particularly sensitive method of determining early changes in the lungs. In the various studies, the following parameters were determined in the lavage fluid or the cells (Pauluhn 2000; Pauluhn et al. 1999 a):

- cell count
- phospholipids in macrophages as evidence of phagocytosis of surfactant phospholipids
- total protein and angiotensin converting enzyme (ACE) to determine increased permeability of the alveolar capillaries
- alkaline phosphatase (AP) to determine the activity of type II pneumocytes
- lysosomal acid phosphatase (ACP) or β-N-acetyl-glucosaminidase (β-NAG) as evidence of the release of enzymes from activated or lysed macrophages
- lactate dehydrogenase (LDH) from cytosol as a marker for cytotoxicity, for example from alveolar macrophages or also passively from serum
- γ-glutamyl transpeptidase (γ-GT) as a marker enzyme for damage or increased activity of Clara cells, including type II pneumocytes
- hydrolysed sialomucins as marker enzymes for the increased activity of secretory cells in the lower respiratory tract
- GSH, to determine the most important nucleophil in the surfactant.

The determination of protein and LDH in the BAL was found to be particularly sensitive for ascertaining the irritating effects of polyisocyanates. Cell proliferation data and changes in the BAL seem to correspond (Kilgour et al. 2002; Pauluhn et al. 1999 a). In the case of **PMDI**, a significant increase in proteins in the BAL of rats was observed immediately after exposure even after single 6-hour exposures to the lowest concentration used of 0.7 mg/m^3. The proteins in the BAL were no longer increased one day after the end of exposure to both 0.7 and 2.4 mg/m^3 (Pauluhn 2000, 2004 a, b). This was regarded as evidence of a short-term, non-adverse influence on the lung surfactants. There were no correlates to cytotoxic effects at this concentration level. Relevant changes in the BAL were observed at 2.4 mg/m^3 and above. Clinical symptoms were found at 8 mg/m^3 and above (Pauluhn 2004 a, b). At this concentration an increase in GSH in the BAL, a typical effect of irritating substances, an increase in cell count and γ–GT, a marker enzyme for damage or increased activity of Clara cells or type II pneumocytes (Pauluhn 2000) and effects on functional respiratory parameters (Pauluhn 2004 b) were observed. At 10 mg/m^3, the incorporation of BrdU in terminal bronchioles, an indication of cell proliferation, was increased (Kilgour et al. 2002). Concentrations of 20 mg/m^3 produced laboured breathing, nasal discharge, incrustations around the nostrils, transient body weight loss and increased lung weights (Pauluhn 2000, 2004 a, b). At 30 mg/m^3 the clinical symptoms were more severe and hypoaesthesia occurred at 100 mg/m^3 (Kilgour et al. 2002).

Summary

After short-term inhalation exposure to MDI and PMDI, the main effect is local irritation in the lower respiratory tract (alveolar region). As the effects of **PMDI** observed at 0.7 mg/m³ are regarded as a homoeostatic reaction because of their rapid reversibility, and therefore not as adverse, this concentration can be considered to be the NOAEC (no observed adverse effect concentration) for single exposures. Adverse effects are observed at 2.4 mg/m³ and above. On the basis of a study in which the lowest investigated concentration of **PMDI** was 3.4 mg/m³, the NOEC (no observed effect concentration) for increased protein concentrations in the BAL was estimated to be 0.5 mg/m³ (Pauluhn 2002 b).

5.2 Subacute, subchronic and chronic toxicity

MDI

Studies with repeated inhalation exposure of rats to **MDI** are summarized in Table 2.

Table 2 Studies with rats with repeated inhalation exposure to MDI

Species, strain, number of animals per group	Exposure, investigation	Findings	References
rat, Wistar, 20 ♀ per group (0.3, 1 mg/m³); 40 ♀ per group (0, 3 mg/m³)	**13 weeks**, MDI: 0, 0.3, 1, 3 mg/m³, 18 hr/day, 5 days/week	**0.3 mg/m³**: no effects **1 mg/m³ and above**: body weight gains ↓, relative lung weights ↑, infiltration of mononuclear cells, goblet cell hyperplasia, erosion of the respiratory epithelium in the upper respiratory tract, hyperplasia of the bronchus-associated lymphatic tissue, inflammatory changes in the lungs **3 mg/m³**: granulocytes and lymphocytes in the BAL ↑, macrophages in the BAL ↓, protein, β-glucuronides and lactate dehydrogenase ↑, changes in lung function	UBA 1991

Table 2 (Continued)

Species, strain, number of animals per group	Exposure, investigation	Findings	References
rat, Wistar, 20 ♀ per group	**24 months**, MDI: 0, 0.2, 0.7, 2.0 mg/m^3, aerosol, MMAD 0.68–0.74 µm; 93.5% of the particles < 4.2 µm), 17 hr/day, 5 days/week	**0.2 mg/m^3 and above**: relative lung weights ↑ (after 3 months, no longer after 12 or 20 months), interstitial fibrosis, monocyte infiltration and pigmented macrophages or with particles, lung function disturbances **0.7 mg/m^3 and above**: bronchioalveolar hyperplasia of the bronchiolar type (alveolar bronchiolization) **2.0 mg/m^3**: proliferation of the alveolar epithelium, inflammatory reactions in the nasal cavity, evidence of pigmented macrophages in the mediastinal lymph nodes, one pulmonary adenoma (see Table 5) unusually high mortality in all groups (including controls) from tumours of the pituitary and mammary glands	UBA 1995

In a 13-week inhalation study, female Wistar rats were exposed to concentrations of **MDI** of 0, 0.3, 1 or 3 mg/m^3 for 18 hours a day on 5 days a week (no other details). Reduced body weight gains and an increase in relative lung weights were found at 1 mg/m^3 and above. At and above this concentration also infiltration of mononuclear cells, goblet cell hyperplasia, erosion of the respiratory epithelium in the upper respiratory tract, hyperplasia of the bronchus-associated lymphatic tissue and inflammatory changes of the lung were observed. At 3 mg/m^3 there was an increase in the total cell count and proportion of granulocytes and lymphocytes, a decrease in the proportion of macrophages in the bronchoalveolar lavage fluid, an increase in protein, β-glucuronides and lactate dehydrogenase, and changes in lung function. No effects were observed at 0.3 mg/m^3 (UBA 1991).

In the subsequent long-term study, female Wistar rats were exposed to **MDI** concentrations of 0, 0.2, 0.7 or 2.0 mg/m^3 (aerosol, MMAD 0.68–0.74 µm; 93.5% of the particles < 4.2 µm) for 17 hours a day on 5 days a week for 24 months. Mortality, particularly from tumours of the pituitary and mammary glands, was increased in all groups (including the control group). After only 19 to 20 months of life (17–18 months of exposure) mortality was 50% and after 24 months 81% in the control group and 87% in all exposure groups. After concentrations of 0.2 mg/m^3, intersti-

tial fibrosis, monocyte infiltration and pigmented macrophages or macrophages with particles were observed as the first effects. There was a concentration-dependent increase in relative lung weights and lung function disturbances. At 0.7 mg/m^3 there was in addition bronchioalveolar hyperplasia of the bronchiolar type (alveolar bronchiolization) and at 2.0 mg/m^3 proliferation of the alveolar epithelium, inflammatory reactions in the nasal cavity and evidence of pigmented macrophages in the mediastinal lymph nodes (see Table 2; see Table 5; UBA 1995). Under the conditions of this study, the LOAEC (lowest observed adverse effect concentration) is considered to be 0.2 mg/m^3.

PMDI

Studies with repeated inhalation exposure of rats to PMDI are summarized in Table 3.

Table 3 Studies with repeated inhalation exposure of rats to PMDI

Species, strain, number of animals per group	Exposure, investigation	Findings	References
rat, Wistar, groups of 17 ♂ and 17 ♀	**14–15 exposures**, PMDI: 0, 1.1, 3.3, 13.7 mg/m^3, MMAD 1.46–1.52 µm, 6 hr/day, 7 or 5 days/week, nose only	**1.1 mg/m^3 and above**: disturbance in phospholipid homoeostasis, BrdU in the terminal bronchioles ↑ **3.3 mg/m^3 and above**: transient tachypnoea, irritation of the lower respiratory tract with evidence of cytotoxicity with plasma extravasation in the BAL, accumulation of alveolar macrophages, slight indications of bronchioalveolar inflammation, intraalveolar surfactant ↑, type II pneumocyte activity ↑ **13.7 mg/m^3** (exposure increased from10 to 16 mg/m^3): marked clinical symptoms (for example nasal discharge, irregular and laboured breathing, dyspnoea, rales), goblet cell hyperplasia in the nose (♂)	Pauluhn et al. 1999 a
rat, Wistar, groups of 10 ♂ and 10 ♀	**14 days**, PMDI: 0, 2.2, 4.9, 13.6 mg/m^3, aerosol, 95% < 5 µm; 6 hr/day, 5 days/week	**2.2 mg/m^3 and above**: relative lung weights ↑ (not significantly) **4.9 mg/m^3**: restlessness and dyspnoea, lung weights ↑ (significantly) **13.6 mg/m^3**: mortality, severe dyspnoea, growth retarded, body weights ↓	Reuzel et al. 1994 a

Table 3 (Continued)

Species, strain, number of animals per group	Exposure, investigation	Findings	References
rat, Wistar, groups of 20 ♂ and 20 ♀	**14 days**, PMDI: 0, 14.1 mg/m^3, aerosol, 95% < 5 µm; 6 hr/day, 5 days/week	**14.1 mg/m^3**: mortality after 13 days: ♂: 15/20 (young animals) and 12/20 (older animals) ♀: 20/20 (young animals) and 1/20 (older animals)	Reuzel et al. 1994 a
rat, Wistar, 42 ♀ per group	**3 weeks**, PMDI: 0, 12.9 mg/m^3, 6 hr/day, 5 days/week, nose only, investigation on days 1, 4 and 11 during exposure and 1 day, 21, 28 and 35 days after the end of exposure	**12.9 mg/m^3**: evidence of airway irritation (reversible), body weight gains ↓ (reversible), lung weights markedly ↑ (reversible; no longer significant from day 25 after the end of exposure), marked effects in BAL (cell count and phospholipid content ↑ with time, normalization within 10 days; protein ↑ on day 1 of exposure to a maximum level; reversible 4 days after the end of exposure), evidence of adaptation during the exposure	Pauluhn 2002 a, b
rat, Alpk:ApfSD, 5 ♀ per group	**4 weeks**, PMDI: 0, 1, 4 or 10 mg/m^3, 6 hr/day, 5 days/week, nose only, investigation 1 day and 30 days after the end of exposure	**1 mg/m^3 and above**: disturbance in surfactant homoeostasis, BrdU incorporation in the terminal bronchioles ↑ (reversible), minimal bronchiolitis (reversible), electron microscopic findings (increase in size of lamellar bodies in type II cells, amorphous surfactant in macrophages ↑, surfactant in the lumen ↑) **4 mg/m^3 and above**: cell proliferation in the terminal bronchioles and the centroacinar region (reversible), findings in BAL (leukocytes ↑, lymphocytes ↑, phospholipids in the cell pellet ↑) **10 mg/m^3**: lung weights (no other details) ↑ (reversible), BAL (total cell count, macrophages, leukocytes, lymphocytes, phospholipids in the cell pellet, total protein, alkaline phosphatase and phospholipids ↑), lung histology (marked bronchiolitis	Kilgour et al. 2002

Table 3 (Continued)

Species, strain, number of animals per group	Exposure, investigation	Findings	References
		and thickening of the centroacinar region and increase in macrophages with inclusion of yellow pigment), findings of electron microscope (interstitium thickened, pronounced increase in size of lamellar bodies in type II cells, surfactant in macrophages ↑, surfactant in the lumen ↑) not reversible	
rat, Wistar, groups of 15 ♂ and 15 ♀	**13 weeks**, PMDI: 0, 0.35, 1.4, 7.2 mg/m^3, aerosol, 95% < 5 µm; 6 hr/day, 5 days/week	**1.4 mg/m^3**: NOAEC **7.2 mg/m^3 and above**: body weight gains ↓ (♂), histological effects in the lung (increase in alveolar macrophages with yellow inclusions partly with slight inflammatory changes, yellowish deposits on the mucous membrane of the respiratory tract)	Reuzel et al. 1994 a
rat, Wistar, groups of 15 ♂ and 15 ♀	**13 weeks**, PMDI: 0, 4.1, 8.4, 12.3 mg/m^3, aerosol, 95% < 5 µm; 6 hr/day, 5 days/week, investigation up to 4 weeks after end of exposure	**4.1 mg/m^3 and above**: no clinical symptoms, body weight gains occasionally ↓ (♂), histological effects in the lung (increase in alveolar macrophages and interstitial macrophage infiltration) and in the mediastinal lymph nodes (macrophages with yellowish inclusions) **8.4 mg/m^3 and above**: moderate dyspnoea, plasma creatinine ↑ (♀), relative lung weights ↑ (♂, ♀), histological effects in the nose (damage to the olfactory epithelium, basal cell hyperplasia; not completely reversible) **12.3 mg/m^3**: marked dyspnoea, mortality, body weight gains consistently ↓ (♂)	Reuzel et al. 1994 a

Table 3 (Continued)

Species, strain, number of animals per group	Exposure, investigation	Findings	References
rat, Wistar, groups of 60 ♂ and 60 ♀	**24 months**, PMDI: 0, 0.2, 1, 6 mg/m³, aerosol, MMAD 0.68–0.74 µm; 93.5% of the particles < 4.2 µm), 6 hr/day, 5 days/week	**0.2 mg/m³**: NOAEC **1 mg/m³ and above**: histological effects in the lungs (increase in alveolar macrophages, local fibrosis, epithelialization of the alveolar duct), mediastinal lymph nodes (macrophages with yellowish inclusions) and nose (degeneration and basal cell hyperplasia of the olfactory epithelium, hyperplasia of Bowman's gland) **6 mg/m³ and above**: clinical symptoms (sniffing noises), absolute and relative lung weights ↑, pulmonary adenomas and adenocarcinomas (see Table 6)	Reuzel et al. 1994 b

In inhalation studies in rats exposed to **PMDI** for 6 hours a day, disturbances in surfactant homoeostasis, evidence of reversible proliferation in the terminal bronchioles, reversible minimal bronchiolitis and electron microscope findings in the lungs were observed after exposure for 2 and 4 weeks to concentrations of 1 mg/m³ and above (Kilgour et al. 2002; Pauluhn et al. 1999 a). The lung weights were slightly increased at 2 mg/m³ and above; the increase was statistically significant at 4.9 mg/m³ and above (Reuzel et al. 1994 a; Gamer et al. 2000). Marked clinical symptoms and mortality were observed at concentrations above 13 mg/m³ (Reuzel et al. 1994 a; Gamer et al. 2000; Pauluhn et al. 1999 a). In studies of the effects of exposure to 12.9 mg/m³ for 3 weeks it was demonstrated that the clinical symptoms of airway irritation, the reduced body weights, the increased lung weights and the effects in the BAL were reversible (Pauluhn 2002 a, b).

In a 13-week inhalation study with daily exposure of male and female rats to **PMDI** for 6 hours, histological effects in the lungs (increase in alveolar macrophages and interstitial macrophage infiltration) and in the mediastinal lymph nodes (macrophages with yellowish inclusions) were found at 4.1 mg/m³; increased relative lung weights, not completely reversible damage to the olfactory epithelium and basal cell hyperplasia were observed at 8.4 mg/m³ and above. Mortality was increased at 12.3 mg/m³. No effects were found after exposure to 0.35 or 1.4 mg/m³ for 13 weeks (Reuzel et al. 1994 a). In a subsequent long-term study with **PMDI** for 24 months, however, histological effects in the lungs, the mediastinal lymph nodes

and the nose could still be observed at 1 mg/m³. In addition, clinical symptoms, increased lung weights, and pulmonary adenomas and adenocarcinomas occurred at 6 mg/m³ (see also Table 6). The NOAEC was 0.2 mg/m³ (Reuzel et al. 1994 b).

The conditions of the long-term inhalation studies in rats with daily exposure to **MDI** for 17 hours (UBA 1995) or daily exposure to **PMDI** for 6 hours (Reuzel et al. 1994 b) were compared with each other, and the histopathological sections of the female animals of both studies were re-evaluated. Among other factors, the cumulative dose and the retained dose of reactive NCO groups per lung weight was calculated (see Table 4). It was found that daily exposure to **MDI** concentrations of 0.2 mg/m³ for 17 hours approximately corresponds to daily exposure to **PMDI** concentrations of 1 mg/m³ for 6 hours (Feron et al. 2001).

Table 4 Comparison of doses in the long-term inhalation studies with **MDI** (UBA 1995) and **PMDI** (Reuzel et al. 1994 b) in Wistar rats and selected results of the re-evaluation of histopathological sections of female animals (Feron et al. 2001)

	PMDI concentration (mg/m³) (Reuzel et al. 1994 b)				**MDI** concentration (mg/m³) (UBA 1995)			
	0	0.2	1.0	6.0	0	0.2	0.7	2.0
total exposure duration (hours)	0	2940	no data	no data	0	8573	no data	no data
cumulative dose (mg × hr/m³)	0	559	2881	17 728	0	1972	6001	17 575
retained dose of NCO groups per lung weight of controls (µmol/g)	0	0.52	3.45	22.19	0	3.65	16.84	54.72
histological findings in the lungs (in %)								
bronchioalveolar hyperplasia	19	17	42	100	10	20	34	66
bronchioalveolar adenomas	0	0	0	3	0	0	0	1
interstitial fibrosis	3	3	32	100	13	79	96	99
metaplasia	2	5	3	3	6	9	9	19
calcifications	8	2	12	64	0	5	1	16
		NOAEC				**LOAEC**		

Summary

In a number of very detailed studies it was shown that also after repeated inhalation the toxicity of MDI and PMDI is determined by the local irritation in the lower respiratory tract. Initial effects are observed after daily exposure to **PMDI** concentrations of about 1 mg/m^3 and above for 6 hours; increasing the exposure duration did not result in a reduction in the concentration that causes the effects. At and above around 1 mg/m^3, there is also an increase in phospholipid precipitates in the alveoli, an indirect indication of a disturbance in the surfactant layer with corresponding sequel reactions (for example, an increase in the cell proliferation rate of type II pneumocytes, the surfactant-producing cells). A NOAEC for **PMDI** of 0.2 mg/m^3 was obtained in a 24-month inhalation study with workplace-relevant daily exposure for 6 hours. In an inhalation study with exposure to **MDI** for 17 hours a day for 24 months, effects on the lungs were observed even at concentrations as low as 0.2 mg/m^3. This concentration corresponds to daily exposure to MDI concentrations of about 1 mg/m^3 for 6 hours. As the effects induced by MDI and PMDI are similar, the NOAEC of 0.2 mg/m^3 obtained for PMDI can also be used for MDI.

5.3 Local effects on skin and mucous membranes

5.3.1 Skin

In earlier studies with **MDI** or undefined MDI, slight to marked irritation and induration of the skin was observed in rabbits. In a more recent study in rabbits carried out according to OECD Test Guideline 404, undiluted **MDI** was found to be severely irritating when applied semi-occlusively for 4 hours. The effects had not subsided in 5 of 6 animals by the end of the 14-day recovery period (EU 2005).

5.3.2 Eyes

In earlier studies with **MDI** or **PMDI**, irritating effects on the rabbit eye were observed. In a more recent study according to OECD Test Guideline 405, undiluted **MDI** was found not to be irritating in rabbits (EU 2005).

5.4 Allergenic effects

5.4.1 Skin

In a maximization test with guinea pigs and in a mouse ear swelling test, **MDI** was found to cause skin sensitization; in a maximization test with **PMDI**, however, sen-

sitizing effects were not found (see documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997; Rattray et al. 1994).

Also in a local lymph node assay (LLNA) in the mouse, which was published only as an abstract, **MDI** had skin-sensitizing effects. This sensitization occurred with an EC_3 (estimated concentration of test substance necessary to obtain a 3-fold increase in proliferation in the LLNA) of 0.09% at a lower concentration than that which produced local irritation (MIC_{10}, that is the minimal concentration to cause local irritation and produce a 10% thickening of the ear in the LLNA: 0.28%; Anderson et al. 2003).

In other LLNAs, **MDI** (0.5% or 1%) produced lymphocyte proliferation or an increase in the weight of lymph nodes (Hayashi et al. 2001; Hilton et al. 1994). Concentrations of 0.03% to 3% or 0.02% to 2%, yielded positive dose-dependent results at and above 0.03% or 0.2%, respectively. The results were negative with 0.02% (Hilton et al. 1995; Plinick et al. 2005).

5.4.2 Respiratory tract

While there is no validated test system available to date to investigate the sensitizing effects of substances on the respiratory tract, some experience from animal studies are available for the respiratory sensitizing effects of diisocyanates. A number of studies of the sensitization potential of MDI in the respiratory tract have been carried out, mostly in guinea pigs. The induction treatment was performed by dermal application, intradermal injection or by inhalation (see documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997; EU 2005; Pauluhn 1994 a, b, 2005; Pauluhn and Mohr 1994; Pauluhn et al. 1999 b, 2000; Selgrade et al. 2006). After intradermal induction (0.0003%–1%) and challenge treatment via inhalation (18–42 or 55 mg/m^3), reactions to the challenge treatment with **MDI** were observed after intradermal induction concentrations of 0.01% and above (Blaikie et al. 1995). After induction via inhalation (2.2 ml/m^3; about 22.9 mg/m^3) and challenge treatment (0.3–1 ml/m^3; about 0.9–10.4 mg/m^3), 63% of the guinea pigs produced a delayed reaction (Griffiths-Johnson et al. 1990). The methods used in these experiments with animals to investigate respiratory allergy resulting from (di)isocyanates are discussed using the example of MDI, among other substances (Pauluhn and Mohr 2005).

To investigate the influence of the route of absorption on respiratory sensitization caused by **MDI**, a comparative study was carried out in guinea pigs with intradermal (0.003%–0.3%), dermal (10%, 30%, 100%) or inhalation induction (19.4 or 23.7 mg/m^3, 3 hours a day, 5 days). The challenge treatment was carried out via inhalation exposure (25.9–44.1 mg/m^3). A hypersensitivity reaction occurred after intradermal induction at 0.03% and above and after dermal induction at 10% and above, whereas no reactions were observed after inhalation induction and inhalation challenge treatment (Rattray et al. 1994).

A comparative study with dermal (150 µl and 75 µl) or inhalation induction (**MDI** concentrations of 28.3 mg/m^3 for 3 hours a day on 5 days, aerosol, MMAD

about 1.6 μm) was carried out in the Brown Norway rat. The inhalation challenge treatment was carried out with 15.7 mg/m^3 for 30 minutes on days 21, 35, 50 and 64 after induction. The lung function was investigated during a period of up to 30 minutes after exposure. To exclude non-specific bronchial hyperreactivity, exposure to methacholine was carried out one day after the challenge treatment with MDI. Two days after inhalation challenge treatment, the weight of the lungs and local lymph nodes were determined in some of the animals, lung lavage was carried out and serum IgE was determined. In the animals with challenge treatment on days 21 or 64, in addition the lungs and lymph nodes were examined histologically. Inhalation induction resulted in laboured breathing and nasal discharge. No treatment-related effects on IgE were demonstrated in either the serum or the lavage fluid. On day 21, effects on lung function were observed after inhalation induction, whereas later challenge treatments did not produce corresponding effects. There was no evidence of non-specific bronchial hyperreactivity. In the dermally induced animals, moderate effects in the lung lavage (increase in neutrophilic leukocytes) and slightly increased lung and lymph node weights, histologically activated lymph node tissue and increased eosinophilic leukocytes in the lungs were found (Pauluhn et al. 2005).

In a subsequent study with Brown Norway rats, induction was carried out dermally with 40 or 150 μl **MDI** (cumulative dose 127.6 and 398 mg/rat) on days 0 and 7. The inhalation challenge treatment was carried out on days 16, 35, 49 and 63 or 69 for 30 minutes with 39 mg/m^3 **MDI** aerosol. In the control group, the challenge treatment did not produce any acute symptoms or delayed changes in lung function, while in the group exposed to MDI transient acute airways symptoms were observed, and there was a delayed change in lung function, which increased in severity during the course of the challenge treatment. The total serum IgE was increased in the high dose group (dermal induction). In the lung lavage—carried out after the last challenge treatment—there was an increase in cell count, LDH, protein, macrophages, neutrophils, lymphocytes and eosinophils in both groups exposed to MDI. Lung weights were increased, and inflammatory changes were observed on histological investigation of the lungs (Pauluhn 2005).

In another study with Brown Norway rats, there was an increase in cytokines (interferon-γ, IL-1α, IL-4, TNFα, GM-CSF, MCP-1) in the bronchoalveolar lavage after dermal induction and inhalation challenge treatment (**MDI** concentrations of 39 mg/m^3 for 30 minutes a day on 4 days) and destruction of the C fibres with capsaicin; the changes in cytokines with intact C fibres were much less pronounced. The differences in the clinical symptoms were slight (Pauluhn and Vohr 2006).

In a study with guinea pigs, in which the inhalation challenge treatment was carried out with two different **PMDI** aerosols (15 or 45 mg/m^3; MMAD 1.7 or 3.8 μm) after intradermal (0.9 mg/animal) or inhalation induction (135 mg/m^3), there were effects on breathing (after induction with large particles and challenge with the smaller particles) and on the influx of eosinophilic granulocytes in the mucosa and submucosa of the airways and in the lymph nodes associated with the lungs. For induction, the strength of the effects was greater with large particles, whereas for

the challenge treatment the effects were more pronounced with the smaller particles (Pauluhn et al. 2000).

Groups of 11 guinea pigs were exposed to **MDI** concentrations of 0, 5, 10 or 20 ppb (0, 0.05, 0.1 or 0.2 mg/m^3) for 6 hours a day on 5 days and the reaction of the trachea to acetylcholine was investigated ex vivo. The reaction of the smooth muscles was more pronounced at and above 10 ppb, while no effects were observed at 5 ppb. To clarify whether the effects were reversible, 2 further groups of 12 guinea pigs were exposed to MDI concentrations of 10 ppb or 20 ppb (0.1 or 0.2 mg/m^3) for 6 hours a day on 5 days a week for 4 weeks and some of the animals were monitored over a recovery period of 8 weeks. The more pronounced reaction of the smooth muscles to acetylcholine observed directly after the end of the exposure was no longer detectable at the end of the 8-week recovery period (Marek et al. 1999).

By means of the LLNA and the determination of the cytokine profile, attempts are being made at present to differentiate between substances allergenic to the skin and the airways. Another attempt to develop a method for the recognition of airway sensitization in experimental animals, is the determination of IgE in the mouse. Also MDI is included in these activities to develop a method. A large number of studies have been published on this subject, which, however, make no contribution to the derivation of a threshold limit value for the workplace, and are therefore not quoted in full here. An introduction to this subject can be found, for example, in Dearman et al. (1992 a, b, 1996), Plinick et al. (2005), and Selgrade et al. (2006).

5.5 Reproductive toxicity

5.5.1 Fertility

The influence of **MDI** or **PMDI** on fertility has, to date, not been investigated. In studies with repeated exposure, no evidence of impairment of the reproductive organs was found.

5.5.2 Developmental toxicity

In a developmental toxicity study, groups of 25 to 26 pregnant Wistar rats were exposed in whole-body chambers to an **MDI** aerosol at concentrations of 0, 1, 3 or 9 mg/m^3 daily for 6 hours from days 6 to 15 of gestation. There were no clinical signs of intoxication; maternal body weight gains were not affected. In all treated groups, however, food consumption was transiently reduced. There was an increase in the relative and absolute lung weights at 9 mg/m^3; these parameters were not determined, however, in the two low concentrations groups. All reproduction parameters (corpora lutea, implantations, pre-implantation and post-implantation losses) were unchanged. The foetal body weights and placental weights were

unchanged, and no evidence of visceral or skeletal malformations or delays in ossification was found. A slight increase in the number of litters with foetuses with asymmetric sternebrae observed at the high concentration was, according to the authors, within the range of known biological variability. However, they did not exclude the possibility of a substance-induced effect. A NOAEC for developmental toxicity was given of 3 mg/m^3 (Buschmann et al. 1996). Also the NOAEC for maternal toxicity was 3 mg/m^3.

In a study of developmental toxicity with **PMDI**, groups of 25 pregnant Wistar rats were exposed in whole-body chambers for 6 hours a day to 0, 1, 4 or 12 mg/m^3 from days 6 to 15 of gestation. Maternal mortality (2/24), reduced body weight gains (–30%), reduced liver weights (–18%) and increased lung weights (+12%) were observed at 12 mg/m^3. Developmental toxicity was likewise observed at 12 mg/m^3. Foetal and placental weights were reduced (–10% and –7%, respectively), and the incidences of skeletal variations and skeletal retardations were slightly increased outside the concentration range of historical control data. There were no maternal or foetal effects up to 4 mg/m^3. The NOAEC for maternal and developmental toxicity was therefore given as 4 mg/m^3 (Gamer et al. 2000).

In a study of transplacental migration after whole-body inhalation exposure to a monomeric **MDI** aerosol, pregnant Wistar rats were exposed for 6 hours on day 19 of gestation to 20 mg/m^3; this single exposure was followed by the collection of maternal blood, amniotic fluid, the foetuses and placentas. After acid hydrolysis, MDA was analytically determined. The highest MDA concentrations were found in the blood (12.5 ng/g), followed by the placenta (8.3 ng/g; 66.4% of the value in whole blood), foetus (5.3 ng/g; 42.4% of the value in whole blood) and amniotic fluid (1.7 ng/g; 13.6% of the value in whole blood) (UBA 1996).

5.6 Genotoxicity

The formulation of MDI with commercially available DMSO, which contains H_2O in the concentrations normally used in the Ames test, leads to the hydrolysis of MDI to MDA and the formation of a large number of undefined degradation products (Gahlmann et al. 1993; Seel et al. 1999). This problem, typical of diisocyanates in mutagenicity tests in vitro, has been confirmed in experiments also for the isomer diphenylmethane-2,4′-diisocyanate (2,4′-MDI), for a mixture of monomeric diphenylmethane diisocyanates (4,4′-MDI, 2,4′-MDI and 2,2′-MDI), and for PMDI (Herbold et al. 1998; Seel et al. 1999). MDI is stable for several hours in ethylene glycol dimethyl ether. Unlike with TDI, with MDI dissolved in ethylene glycol dimethyl ether no MDA is formed on contact with the test medium of the Ames tests (Seel et al. 1999).

The majority of studies of the genotoxicity of MDI and PMDI are listed in the documentation from 1992 (documentation “4,4′-Methylene diphenyl diisocyanate

(MDI) and "polymeric" MDI (PMDI)" 1997). Below, only those publications which have appeared since then are mentioned.

5.6.1 In vitro

As explained above, the earlier studies with DMSO cannot be included in the evaluation for methodological reasons.

In a study using human lung epithelial cells (A549), **MDI** was incubated in ethylene glycol dimethyl ether for 2 hours, and the cells were cultured further for 6, 22 or 70 hours. Double strand breaks with simultaneous cytotoxicity were observed at 100 µmol/l and above. The concentration at which survival was reduced by 50% (LC_{50}) was 200 µmol/l. At 300 µmol/l and above, hydrolysis of MDI occurred and polyurea was formed. The formation of DNA fragments was time-dependent, with smaller fragments the longer the cultivation time. This suggests an indirect enzymatic process during cellular lysis. The irregular aggregates of chromatin found in the cell nuclei were interpreted as being signs of necrotic processes. Overall, no evidence of the formation of MDI-induced DNA crosslinks can be derived from the study (Vock et al. 1998).

The DNA binding potential of **MDI** and an **MDI mixture** (60% MDI, 30% various triisocyanates, 10% undefined diisocyanates and hexamethylene diisocyanate) was investigated in vitro in calf thymus DNA. No evidence of DNA crosslinks or an increase in DNA fragmentation was found with MDI or the MDI mixture (Peel et al. 1997).

By means of ^{32}P postlabelling, it was demonstrated that **MDI** in acetone binds to isolated rat liver DNA; this took place to a much smaller extent than the binding of MDI to isolated nucleotides (Vock et al. 1995).

In an in vitro micronucleus test with V79 cells, **MDI**, MDI cysteine conjugate and MDI-GSH conjugate were formulated in DMSO. Concentrations of 50 µg/ml (MDI cysteine) and 100 to 500 µg/ml or 1000 µg/ml were investigated. There was no increase in micronuclei in the case of MDI, whereas the number of micronuclei was increased with the two conjugates at and above 50 and 100 µg/ml, respectively. However, no dose–effect relationship was found with the MDI cysteine conjugate. The MDI-GSH conjugate produced an increase in cells in the metaphase and intracellular precipitates (Zhong and Siegel 2000). In a further study, the increase in micronuclei produced by the conjugates was confirmed and a disturbance of the mitotic spindle was demonstrated by staining the kinetochores (Zhong et al. 2001). As DMSO was used as the solvent in both studies, solvolytic effects cannot be excluded, however.

5.6.2 In vivo

In rats, MDI was not found to bind to DNA in the lungs as the target organ of MDI toxicity and in systemic organs such as the liver, kidneys and bladder after inhala-

tion exposure to 0, 0.3, 0.7 or 2 mg/m^3 for 17 hours a day for one year in an investigation using ^{32}P postlabelling with a detection limit of 0.7 to 3 adducts per 10^9 nucleotides, whereas MDA produced DNA adducts in the liver after oral administration. MDI was found to bind to DNA to a very small extent only in the olfactory epithelium. Values of 5, 9 and 10 adducts per 10^{10} nucleotides at a detection limit of 4 adducts per 10^{10} nucleotides were found for the three exposure concentrations (Vock et al. 1996). The toxicological relevance of this observation is questionable as MDI causes degeneration in the olfactory epithelium only in high concentrations.

MDI was not found to bind to DNA in the lungs, liver, kidneys and bladder in an investigation using ^{32}P postlabelling with a detection limit of 2 adducts per 10^{10} nucleotides after **MDI** in acetone was applied dermally (non-occlusive) to the shaved back of female Wistar rats for 90 minutes in doses of 9 mg/animal. In the skin, the formation of DNA adducts with 7 adducts per 10^8 nucleotides was observed; far fewer DNA adducts were formed than with 7,12-dimethyl-benz[*a*]anthracene (1000-fold difference) (Vock et al. 1995). Also after the dermal application of **MDI** in acetone in doses of 2.5 to 6.9 mg/rat, a low level of binding to DNA, with 3 to 4 adducts per 10^7 nucleotides, was observed in the skin by means of ^{32}P postlabelling. As a result of the extensive binding of MDI to proteins, the authors assumed a substantial fraction of the DNA adducts to be simulated by contamination with proteins, that is, they are non-specific. In the liver, very slight covalent binding was determined with a covalent binding index (CBI) of below 0.1 µmol adducts/mol DNA nucleotides, which was only slightly above the detection limit (Vock and Lutz 1997).

In an in vivo micronucleus test, Brown Norway rats were exposed by inhalation in whole-body chambers to concentrations of monomeric **MDI** of 0, 7 or 113 mg/m^3 for one hour a day once a week for 3 weeks. A dose-dependent increase in micronuclei in the bone marrow smear was found on day 7 after the end of exposure: 1.5% and 4.1% compared with 0.95% in the controls. The proportion of polychromatic erythrocytes was unchanged (Siegel et al. 1999; Zhong and Siegel 2000). However, the study is of questionable usefulness as a result of methodological shortcomings such as the absence of positive controls and historical control data, evaluation only after 7 days but not before, and the problematic generation of the aerosol at 130°C.

In contrast, negative results were obtained in a valid micronucleus test in Brown Norway rats with an identical study design (whole-body exposure to MDI concentrations of 9.2 and 118 mg/m^3), but three different methods of analysis of the MDI aerosol, the additional use of a nose-only mode of exposure to MDI concentrations of 110 mg/m^3, two positive controls (cyclophosphamide and colcemid), and additional earlier bone marrow smears carried out according to the guidelines (24 and 48 hours after the end of exposure). Toxic effects such as difficult, irregular and slow breathing, serous nasal discharge and increased absolute and relative lung weights were observed in the two high concentration groups (Pauluhn et al. 2001).

The positive results obtained in the study of Zhong and Siegel (2000) are therefore regarded as not relevant.

5.7 Carcinogenicity

The results of studies of the carcinogenicity of MDI are given in Table 5, those for PMDI in Table 6. Also after long-term exposure, the lungs are the target organ.

MDI

In a long-term study, female Wistar rats were exposed for 24 months to an **MDI** aerosol at concentrations of 0, 0.2, 0.7 or 2 mg/m^3 for 17 hours a day on 5 days a week. At and above 0.2 mg/m^3, there was a concentration-dependent increase in lung weights (after 3 months, but no longer after 12 or 20 months) and interstitial fibrosis, monocyte infiltration, macrophages containing particles and disturbed lung function were observed. At and above 0.7 mg/m^3, alveolar bronchiolization occurred, and at 2 mg/m^3 inflammatory changes in the lungs and nasal cavity, delayed lung clearance, interstitial and peribronchial fibrosis, and proliferation of the alveolar epithelium were observed; in one case a pulmonary adenoma occurred (see Table 5; see Section 5.2; UBA 1995). In this study, the unusually high mortality of the animals in all groups, including the control group, from tumours of the pituitary gland is problematical because the animals may have not survived long enough to develop substance-specific tumours.

PMDI

In a long-term study with daily exposure to a **PMDI** aerosol for 6 hours, pulmonary adenomas occurred at the highest concentration of 6 mg/m^3 in 6 of 60 male animals and 2 of 60 female animals, and one adenocarcinoma in the lung of a male animal. As pulmonary adenomas and adenocarcinomas are rare in Wistar rats, the tumours are regarded as treatment-related. Irritation in the nose and lungs and reactions of the mediastinal lymph nodes occurred at concentrations as low as 1 mg/m^3 and above in a concentration-dependent manner. The local irritation (cytotoxicity) with subsequent hyperplasia of type II cells followed by the formation of adenomas and adenocarcinomas in the lungs was suggested as the mechanism of tumour formation (see Figure 2) (see Table 6; see Section 5.2; Reuzel et al. 1994 b).

Mechanism of lung tumour formation

As explained in Section 2, a non-genotoxic mechanism is assumed to be the cause of the formation of lung tumours from MDI and PMDI. This is supported by the tumour spectrum with a low incidence of mainly benign adenomas, the absence of local invasive growth and the presence of merely microscopic changes towards the end of the study. The findings of long-term inhalation studies with MDI and PMDI are interpreted as non-specific irritation with corresponding typical sequel reac-

Table 5 Studies of the carcinogenicity of MDI

Author:	UBA 1995
Substance:	**MDI**, technical-grade product (minimum purity 99.5%), aerosol (MMAD 0.68–0.74 µm; 93.5% of the particles < 4.2 µm)
Species:	rat, Wistar (Crl:[Wi] Br), groups of 80 ♀
Administration route:	as aerosol (MMAD 1.03–1.06 µm)
Concentration:	0, 0.2, 0.7 or 2 mg/m^3 nominal (analysed concentration: 0, 0.23, 0.7 and 2.05 mg/m^3)
Duration:	24 months, 17 hours a day, 5 days a week
Toxicity:	unusually high mortality in all groups (including controls) from tumours of the pituitary and mammary glands 0.2 mg/m^3 and above: relative lung weights increased, interstitial fibrosis. monocyte infiltration and pigmented or particle-laden macrophages, disturbance in lung function 0.7 mg/m^3 and above: bronchioalveolar hyperplasia of the bronchiolar type 2 mg/m^3: proliferation of the alveolar epithelium, inflammatory reactions in the nasal cavity, evidence of pigmented macrophages in the mediastinal lymph nodes

Non-neoplastic findings and tumours:

		Exposure concentration (mg MDI/m^3)			
		0	0.2	0.7	2
Number of animals (n)	♀	80	80	80	80
Lungs					
alveolar cell hyperplasia (n)	♀	2	8	12	21*
macrophages with particles (n)	♀	0	52*	70*	78*
interstitial fibrosis (n)	♀	4	51*	73*	77*
monocyte infiltration (n)	♀	11	24*	48*	73*
pigmented macrophages (n)	♀	0	6*	9*	14*
cholesterol granules(n)	♀	0	4	1	11*
alveolar bronchiolization (n)	♀	3	6	14*	41*
mineralized deposits (n)	♀	1	6	4	11*
adenomas (n)	♀	0	0	0	1

* $p < 0.05$

Table 6 Studies of the carcinogenicity of PMDI

Author:	Reuzel et al. 1994 b
Substance:	PMDI, technical-grade product (containing 44.8%–50.2% MDI)
Species:	rat, Wistar (Cpb:WU), groups of 70 ♂, 70 ♀
Administration route:	as aerosol (MMAD 0.68–0.74 µm; 93.5% of the particles < 4.2 µm)
Concentration:	0, 0.2, 1 or 6 mg/m³ nominal (analysed concentrations: 0, 0.19, 0.98 and 6.03 mg/m³)
Duration:	24 months, 6 hours a day, 5 days a week
Toxicity:	0.2 mg/m³: NOAEC 1 mg/m³: histological effects in the lung, mediastinal lymph nodes and nose 6 mg/m³: clinical symptoms (sniffing noises), absolute and relative lung weights increased
Non-neoplastic findings and tumours:	

		Exposure concentration (mg PMDI/m³)			
		0	0.2	1	6
Number of animals	♂	60	60	60	60
	♀	60	60	60	60
Surviving animals	♂	38 (63%)	38 (63%)	42 (70%)	36 (60%)
	♀	41 (68%)	42 (70%)	48 (80%)	50 (83%)
Lungs					
macrophages with yellow pigment	♂	0	3	21**	60**
	♀	0	1	23**	59**
local fibrosis	♂	1	0	9*	44**
	♀	0	0	4	48**
alveolar duct epithelialization	♂	1	0	8*	54**
	♀	0	0	8*	57*
alveolar bronchiolization	♂	1	1	2	12**
	♀	2	3	3	14**
mineralized deposits	♂	0	1	1	13**
	♀	0	0	0	24**
pneumonitis	♂	13	13	17	28
	♀	3	4	3	2
adenomas	♂	0	0	0	6*
	♀	0	0	0	2
adenocarcinomas	♂	0	0	0	1
	♀	0	0	0	0

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

tions in the rat lung. Results from animal experiments confirm that the buffer capacity of the surfactant system is exceeded after PMDI aerosol concentrations of about 1 mg/m^3 and above (exposure duration 6 hours a day). It is noteworthy that the NO(A)EC and LO(A)EC from short-term and long-term inhalation studies with PMDI largely agree, which emphasizes the non-cumulative character (buffer capacity of the surfactant system) of the effects of MDI in the lungs. At the same time, this mode of action explains how a longer period of daily exposure (for example 17 or 18 hours a day) with the resulting shortened reconstitution phase leads to increased effects, and that the occurrence of assumedly preneoplastic changes is more likely.

6 Manifesto (MAK value, classification)

For methodological reasons, studies of the genotoxicity of MDI in vitro are of only limited usefulness and do not provide any clear picture. The positive findings are probably produced in vitro by degradation or reaction products of MDI when using DMSO as the solvent. After long-term inhalation exposure, MDI was not found to bind to DNA in the lung, the target organ of MDI toxicity. No genotoxic activity could be demonstrated in vivo in a valid micronucleus test with inhalation exposure up to the highest tested toxic concentration of 118 mg/m^3. Relevant genotoxic effects of MDI and PMDI are therefore not to be expected, and classification in a category for germ cell mutagens is not necessary.

Preneoplastic and neoplastic changes in the lungs were found in rats after long-term exposure to MDI or PMDI aerosols. All available findings indicate a pathomechanism induced by chronic cell proliferation. The formation of the genotoxic and, after oral administration, hepatocarcinogenic MDA cannot be completely excluded. However, the available toxicological data provide no evidence of a relevant contribution of MDA to the toxicity of MDI after inhalation exposure, so that it is thought to be of only minor importance. MDI and PMDI in the form of inhalable aerosols are therefore classified in Carcinogen Category 4.

The tumour-inducing mechanism in the rat is activated only when exposure concentrations are used which produce marked local irritation in the rat even after only short-term exposure for 6 hours. This has been demonstrated in short-term inhalation studies with corresponding changes in the bronchoalveolar lavage and the proliferation of bronchioalveolar epithelial cells. These findings from inhalation studies with short-term exposure thus correspond to those from continuous lifetime exposure. The respiratory irritation after short-term exposure occurs at concentrations approximately 10 times lower than those that cause tumour formation in the respiratory tract. Thus, when irritation is avoided, no carcinogenic effects are to be expected.

Breathing difficulties in workers, although not increased to a significant extent, were observed at MDI concentrations of 0.1 mg/m^3. For the induction of specific airway hyperreactivity, exposures above 0.2 mg/m^3 or intensive skin contact are of importance. Concentrations of 0.05 mg/m^3 did not produce effects (see documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997). In long-term inhalation studies with rats, MDI caused toxic effects on the lungs after very intense exposure (17 hours/day) to concentrations of 0.2 mg/m^3 and above, while corresponding effects occurred after exposure to PMDI concentrations of 1 mg/m^3 and above for 6 hours a day. The NOAEC for PMDI is 0.2 mg/m^3; the NOAEC for MDI is assumed also to be 0.2 mg/m^3 after daily 6-hour exposure. Overall, the experimental findings obtained since the documentation of 1992 provide no evidence that the present MAK value of 0.05 mg/m^3 for inhalable MDI aerosol, which was based on experience in humans, should be modified. As reactive NCO groups are attached to both MDI and PMDI to the same extent, and only slight differences between the biological effects of the two substances were observed, the MAK value of 0.05 mg/m^3 now applies also for PMDI.

The effects of MDI and PMDI on the respiratory tract are dependent above all on the concentration level. As described in the supplement of 2000 (supplement "Diphenylmethan-4,4′-diisocyanat (MDI)" 2000, not yet available in English), exposure to concentrations above 0.2 mg/m^3 or intensive skin contact are of importance for the induction of specific hyperreactivity of the airways. Therefore, temporary increases in peak concentrations should be avoided. Peak Limitation Category I with an excursion factor of 1 and a momentary value of 0.1 mg/m^3 has therefore been retained for MDI and applies now also for PMDI.

As described in the supplement of 1995 (supplement "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 2000), both MDI and PMDI are respiratory allergens in humans. This has been confirmed in more recent reports with exposure to MDI. The designation with "Sa" (for substances which cause sensitization of the airways) for MDI and PMDI has therefore been retained. As sensitization of the skin by MDI has been demonstrated in animal experiments, and as the induction of contact allergy cannot be excluded for PMDI, also designation with "Sh" (for substances which cause sensitization of the skin) for MDI and PMDI has been retained.

The available studies indicate that, after dermal exposure, MDI and PMDI mainly react with molecular components of the skin, and thus only very small quantities are absorbed systemically in unchanged form. In humans, intensive skin contact with MDI can, however, play a role in the induction of specific hyperreactivity of the airways (documentation "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 1997; supplement "4,4′-Methylene diphenyl diisocyanate (MDI) and "polymeric" MDI (PMDI)" 2000;). As technical-grade PMDI contains a considerable amount of monomeric MDI, similar effects are to be assumed. As the prevention of respiratory sensitization was one of the decisive criteria for establishing the MAK value, and this critical effect can also be caused by skin contact, both

MDI and PMDI are designated with an "H" (for substances which can be absorbed through the skin).

In some studies with rats, slight embryotoxic effects occurred in the range of maternal toxicity at 9 mg/m^3 with MDI and 12 mg/m^3 with PMDI. The irritation causes lung effects (an increase in lung weights and a disturbance in lung function); this is regarded as the cause of non-specific growth defects (induced by maternal stress or hypoxaemia). The NOAEC for developmental toxicity is about 3 mg/m^3 for MDI and about 4 mg/m^3 for PMDI. When the MAK value of 0.05 mg/m^3 is observed, no embryotoxic effects are to be expected. MDI and PMDI are therefore classified in Pregnancy Risk Group C.

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