4,4'-Methylene diphenyl isocyanate (MDI) and "polymeric MDI" (PMDI)* S

	MDI	"polymeric MDI"
Classification/MAK value:	**0.005 ml/m³ (ppm) 0.05 mg/m³ Section IIIB MAK List 1992**	**Section IIIB MAK List 1992**
Classification dates from:	**1992**	**1992**

Synonyms:

bis(p-isocyanatophenyl)methane
bis(1,4-isocyanatophenyl)methane
p,*p*'-diisocyanatodiphenylmethane
4,4'-diisocyanatodiphenylmethane
diphenylmethane-*p*,*p*'-diisocyanate
diphenylmethane-4,4'-diisocyanate
isocyanic acid methylene di-*p*-phenylene ester
methylenebis(4-phenylene isocyanate)
methylenebis(*p*-phenylene isocyanate)
p,*p*'-methylenebis(phenyl isocyanate)
4,4'-methylenebis(phenyl isocyanate)
methylene bisphenyl isocyanate
p,*p*'-methylenediphenyl diisocyanate
4,4'-methylenediphenyl diisocyanate
methylenedi-*p*-phenylene diisocyanate
methylenedi-4-phenylene diisocyanate
4,4'-methylenediphenylene isocyanate
methylene di(phenylene isocyanate)

Chemical name (CAS):

MDI: 1,1'-methylenebis(4-isocyanatobenzene)

PMDI: isocyanic acid polymethylenepoly-phenylene ester

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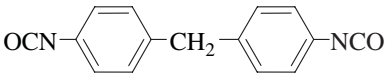
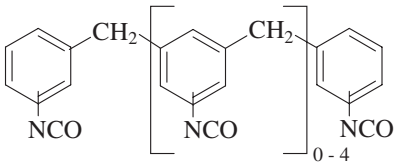
* "Polymeric MDI" (PMDI) is a technical grade MDI which contains 30 to 80 % w/w 4,4'-methylene diphenyl isocyanate; the rest consists of MDI oligomers and MDI homologues (see Note).

	MDI	**PMDI**
CAS number:	101-68-8	9016-87-9
Structural formula:		
Molecular formula:	$C_{15}H_{10}N_2O_2$	$C_{15}H_{10}N_2O_2$ to $C_{47}H_{30}N_6O_6$
Molecular weight:	250.3	250.3 –778
Melting point:	39.5°C	< 0
Boiling point:	210°C at 7 hPa	> 210°C at 7 hPa
Vapour pressure at 20°C:	$< 10^{-5}$ hPa	$< 10^{-5}$ hPa
1 ml/m^3 (ppm) =	**10.4 mg/m^3**	**–**
1 mg/m^3 =	**0.096 ml/m^3 (ppm)**	**–**

Note

4,4'-Methylene diphenyl isocyanate is produced in two grades of purity (Schauerte 1983):

– pure monomeric 4,4'-methylene diphenyl isocyanate (MDI) (CAS number 101-68-8, EC number 615-005-00-9), at temperatures up to 38 °C a whitish-yellow solid which is used in the industrial production of special plastics, varnish and glue components, moulding elastomers and "MDI prepolymers" (reaction products with polyester or polyether polyalcohols containing an excess of MDI) and which accounts for about 10 % to 15 % of all MDI used, and

– technical grade, so-called "polymeric MDI" (PMDI) (CAS number 9016-87-9, EC number 615-005-01-6), dark brown liquids at room temperature which contain between 30 % and 80 % MDI together with higher molecular weight MDI homologues and which are used in numerous industrial plants and workshops, mostly for manufacture of various polyurethane mouldings and products by addition polymerization with polyester or polyether polyalcohols at room temperature. Special applications include core sand binding, chip board production, reinforcement of rock formations in mines.

The production processes for monomeric MDI and PMDI yield products contaminated with the relatively volatile substance, phenylisocyanate. These days, however, the level of phenylisocyanate is generally well below 0.005 % w/w and so is irrelevant for the assessment of the effects of these substances on occupational health.

The spontaneous chemical reaction of the various kinds of MDI with primary or secondary amines or substances with -COOH, -OH or -SH groups is exothermic and complete within seconds or minutes. In humid air, MDI reacts with water vapour and releases CO_2 slowly to form a solid surface layer of polyurea.

The vapour pressure of all kinds of MDI is extremely low. The concentration of MDI in air saturated with monomeric MDI vapour at 20 °C is given as 0.05 mg/m^3

(5 ppb) (Brochhagen and Schal 1986). Thus, in the workplace air at this temperature, MDI concentrations which are less than or equal to 0.05 mg/m^3 (5 ppb) are present as vapour; at higher concentrations some of the substance must be in aerosol form. The aerodynamic diameter of condensed aerosol particles lies between 0.5 and 1.5 µm (Bennett 1991, Rando 1985). It has been suggested that exposure to the different physical forms of MDI has different toxicological effects: whereas inhalation of MDI vapour produces relatively uniform exposure of the whole respiratory tract, after inhalation of an MDI aerosol the regional deposition of the substance will depend on the particle size spectrum and is not expected to be uniform, producing locally more severe toxic effects. The various physical states of MDI must be taken into account during analysis of the concentrations in the workplace air (including sampling); recent systematic studies have shown that numerous other parameters also make such analyses exceedingly difficult (Appelman and Behlau 1982, Fus 1985, Keller 1985, Reuzel *et al* 1986a, Rosenberg and Pfäffli 1982). These results demonstrated that because of inadequacies in the methods, MDI concentrations in the workplace air have regularly been underestimated by 10 % to 50 % in the past, especially when vapour and aerosol or monomeric MDI and PMDI were present simultaneously (Booth 1984, Dharmarajan 1979, Fus 1985, LeBeau 1989, Rosenberg and Pfäffli 1982).

The question of whether MDI vapour or aerosol can hydrolyse in humid air to yield **4,4'-diaminodiphenylmethane (DDPM)** is relevant in the assessment of the carcinogenic potential of MDI. DDPM is classified in category IIIA2 in the "List of MAK and BAT Values": it is carcinogenic in the rat and the mouse and mutagenic in various test systems (see 4,4'-Diaminodiphenylmethane, Volume 7 of this series). In studies aimed specifically at answering this question – both a long-term study (Appelman *et al.* 1986) and a study of chip-board production (Giersig 1989) – no DDPM was detected although the PMDI concentrations ranged up to 5 mg/m^3 (the detection limit for DDPM in air was 5 µg/m^3). This result has been accounted for as follows: DDPM is formed only slowly at neutral pH and reacts rapidly with excess MDI to yield oligoureas and polyureas; MDI aerosols can form a shell of polyurea on the surface of the droplets and this shell prevents further reaction of the enclosed MDI (Mann 1987).

The only situation in which amines can be formed is during decomposition of fully polymerized polyurethanes at temperatures above 250 °C or during gamma irradiation when breakdown of isocyanates can lead to the formation of low levels of the corresponding amines (Renman 1976, Rosenberg and Savoleinen 1986, Shintani and Nakamura 1989).

1 Toxic Effects and Modes of Action

Occupational exposure to 4,4'-methylene diphenyl isocyanate (MDI) affects mainly the respiratory tract; the substance causes irritation of the eyes and respiratory passages and has adverse effects on lung function. This must be distinguished from the bronchial or alveolar hypersensitivity caused by the substance – with or without demonstrated effects on immunological parameters (sensitization). Dermal sensitization is unusual.

Bronchial sensitization has been observed in animal studies after intradermal injection of monomeric MDI; antibodies of IgE type were detected. Marked contact allergies were also seen.

After intradermal injection of PMDI in animal studies, antibodies of IgE type have been demonstrated; contact allergies could not be induced.

Only some of the mutagenicity studies available to date for monomeric MDI and PMDI are usable in the present assessment. The choice of solvent and the method of adding dissolved MDI to the aqueous test medium seem to have a decisive effect on the test result. It is therefore not clear at present how a positive result for the mutagenicity of MDI should be interpreted.

In a long-term inhalation study with rats exposed to PMDI aerosol, concentrations of 6 mg/m^3 air led to chronic inflammatory lesions in the respiratory tract with an increased incidence of lung adenomas and one carcinoma. Local irritation was discussed as the mechanism of tumour induction.

The reproductive toxicology of MDI has not yet been investigated.

1.1 Pharmacokinetics

The details of the metabolism of MDI in man are not known. In the urine of workers exposed to PMDI at concentrations below the then valid MAK value of 0.01 ml/m^3, neither free nor acetylated DDPM was found; only after complete acid hydrolysis of the urine could DDPM be detected, apparently after release from polyureas, urethanes and other metabolites (Lenaerts-Langanke 1992, Lewalter 1991). Conjugation with erythrocytes was not found in these workers (Lewalter 1991).

After intramuscular injection of a dose of about 443 µg/kg body weight of radioactively labelled monomeric MDI dissolved in benzene into Sprague-Dawley rats, low levels of radioactivity were found in the blood after one hour and maximum levels after 25 hours. In some rats, radioactivity could still be detected 360 hours after the injection. In urine and faeces, the maximum levels of radioactivity were found in the 48-hour to 72-hour collection period. A total of 25 % of the administered dose was excreted within 120 hours after the injection (no other details) (Istin 1976).

In another study, 12 male Sprague-Dawley rats inhaled radioactively labelled monomeric MDI aerosol for 15 minutes and were then observed for 96 hours (Istin 1977). Radioactivity in the blood reached a maximum after only 0.25 hours and was detectable until 96 hours after exposure. Most of the radioactivity was excreted in the faeces, with a maximum in the 12-hour to 48-hour collection period whereas, in the urine, radioactivity was detected earlier, in the first 6-hour collection period. After 4 days, a total of 70 % of the absorbed radioactivity had been excreted in urine and faeces.

Autoradiography of the radioactivity distribution in the various organs revealed that the highest concentration after 0.25 hours was in the digestive tract followed by lungs, muscles, blood, skin and liver and after 96 hours in muscles, lungs, blood, digestive tract, skin and liver.

After oral administration of MDI to rats, acetylation products were identified and adduct formation with haemoglobin described (Schütze et al. 1990); as in feeding studies with toluylene diisocyanate (TDI) (NTP 1986), this may be ascribed to amine (DDPM) formation in the stomach contents. This observation is not considered to be of significance for occupational health.

The breakdown pathways of MDI in man and experimental animals are not known in detail; in particular, it has not been demonstrated unambiguously that the free diamine (DDPM) is formed.

Adduct formation with haemoglobin has been described in animals given oral doses of the substance, and also the excretion of acetylation products. In persons exposed to MDI, conjugation with erythrocytes was not detected nor excretion of free or acetylated DDPM in the urine.

There are no studies of the pharmacokinetics of PMDI.

2 Effects in Man

2.1 Acute irritation of eyes and airways

A report from the early years of PMDI application (Longley 1964) described how the aerosol mixture being used to apply a rigid foam insulation to the insides of railway carriages was blown by the wind to a group of twelve workers standing 20 to 40 metres away. Within a few hours, all twelve developed symptoms such as pain behind the eyes, nasal discharge, retrosternal soreness, constriction of the chest, coughing and headaches. The symptoms regressed completely within a few days.

According to another report (Fitzpatrick *et al.* 1964), "vapour concentrations of 56 ppb" during spraying of a PMDI/polyol mixture did not cause irritation of workers using respirators with particle filters. (Since the maximal (saturated) vapour concentration of MDI at 20 °C is about 5 ppb (0.05 mg/m^3), either the temperature at the sampling site was higher or the MDI was mostly in the form of an aerosol; how much of this aerosol was retained in the mask filters is unclear.)

After direct contact of the eyes with PMDI, transient blepharo-conjunctivitis is observed; on the skin the substance causes itching erythema (Bertrand *et al.* 1984).

2.2 Effects on lung function

As may be seen in Table 1, one research group in particular has carried out repeated and intensive studies of the lung function of persons exposed to PMDI. The first cross-sectional study (Cavelier *et al.* 1977, Pham *et al.* 1978) involved 235 exposed and 83 non-exposed persons (214 men, 104 women) in two foam plastics plants. The persons had been exposed to isocyanates for 1 to 7 years (on average 3 $^1/_2$ years) and some of them also to toluylene diisocyanate (TDI) in earlier years. The PMDI concentrations (8-hour averages?) at the workplaces were "mostly less than 20 ppb" (0.2 mg/m^3) but "values up to 87 ppb" (0.9 mg/m^3) were also found (colorimetry). In addition the persons were exposed to tertiary aliphatic amines, stripping and expanding agents, polyvinyl chloride vapour and other products. Respiratory symptoms were found in the exposed persons more often than in the controls; a significant correlation with exposure was

found only for bronchitis in women. Some significant dose-response relationships were seen for spirometry values and the CO transfer factor in men. There were, however, markedly more smokers among the exposed male persons than among the control persons.

Five years later, about half of the workers were examined again (n = 159; 42 persons with low-level exposure, 34 with higher level exposure, 23 persons who had changed their jobs because of respiratory symptoms and 60 control persons) (Pham *et al.* 1986, 1988). The PMDI concentrations at the workplace (8-hour average values, personal samplers) at the later analysis had sunk to "less than 5 ppb" (0.05 mg/m^3) with the exception of one workplace. In the cross-sectional analysis, bronchitis and dyspnoea were more frequent in the exposed persons than in the controls. Some lung function parameters (VC % [1], FEV_1 % [2], TCO % [3]) were significantly lower in the men with high level exposure than in the controls. The long-term development of clinical symptoms was not significantly different in the different exposed groups. At the follow-up examination after 5 years, the average spirometry values were higher in all subgroups than they had been in the original group. When the average later spirometry values in the subgroups were compared with the original average values determined in the same persons 5 years earlier, the differences were not correlated with exposure. Only for TCO % was the five-year difference for the persons with higher exposure levels significantly different from the control value. At the follow-up examination, the lung function of the workers who had changed their jobs because of respiratory symptoms was better than that of the persons who had been continuously exposed; this could be associated with differences in smoking habits. In any case, this observation contradicts an earlier suggestion of the authors that the observed deterioration of lung function could be a sign of pulmonary fibrosis.

In another cross-sectional study, 38 employees of a plant using PMDI to manufacture plastic foam were compared with 38 controls (in both cases, 25 men and 13 women with matched smoking habits) (Martin *et al.* 1982). The average period of employment was five years; the exposure concentrations in the years immediately preceding the study were "under 20 ppb" (0.2 mg/m^3) (analytical method not specified). Asthmatic symptoms and lacrimation at work were reported by the exposed persons significantly more frequently than by the controls. The average spirometry values of the exposed persons were all higher (!) (but not significantly) than those of the control persons.

In an MDI-producing plant with 109 employees who had worked there for years at MDI concentrations "almost always under 20 ppb" (0.2 mg/m^3; nitro reagent method) (Diller and Herbert 1982), cross-sectional analysis revealed a slight (not significant) reduction in the average values of FVC % [4] and FEV_1 % in the group exposed to higher concentrations compared with values for the control group of 83 persons.

[1] VC vital capacity; % expressed as a percentage of the normal value
[2] FEV1 forced expiratory volume in 1 second
[3] TCO CO transfer factor
[4] FVC forced vital capacity

Table 1. Lung function studies in workers exposed to MDI

Work process	MDI concentrations	Other exposures	Size of cohort exposed (control)	Results	References
plastic foam production	"mostly < 0.2 mg/m^3, sometimes up to 0.9 mg/m^3" (8 h?)	in some cases previous exposure to TDI; simultaneous exposure to stripping agents, VC and other products	235 (83)	respiratory symptoms in workers exposed for 1-7 years more frequent (mostly not significantly); dose-dependent adverse effects on lung function parameters (sometimes significant), especially in male heavy smokers	Cavelier *et al.* 1977, Pham *et al.* 1978
plastic foam production	previously up to 0.9 mg/m^3, "recently < 0.05 mg/m^3"		99 (60)	in cross-sectional analysis in exposed workers more frequent (not significantly) respiratory symptoms and (sometimes significantly) impaired lung function; in 5-year longitudinal study no clear dose-response relationship; significant reduction in TCO % only in workers exposed to high concentrations.	Pham *et al.* 1986, 1988
plastic foam production	"< 0.2 mg/m^3"		38 (38)	after average exposure periods of 5 years, asthmatic symptoms and lacrimation significantly more frequent in exposed persons but spirometry values (not significantly) more frequently in the normal range than in the control group	Martin *et al.* 1982
MDI production	"mostly < 0.2 mg/m^3"	phenylisocyanate, phosgene	109 (83)	spirometry values slightly (not significantly) lower in the persons exposed to higher concentrations; in 2-year longitudinal study no trend-like differences	Diller and Herbert 1982

Table 1. (continued)

Work process	MDI concentrations	Other exposures	Size of cohort exposed (control)	Results	References
insulation foam production	"mostly < 0.1 mg/m^3"		20 (36)	after $1^1/_2$-year exposure, more frequent work-related symptoms in the exposed group than in the controls; spirometry values slightly (not significantly) lower than in the controls	Kolmodin-Hedman *et al.* 1980
plastic foam production	< 0.04 mg/m^3 (20-60min)	previously TDI (max. 15 ppb, 90% <5ppb) (in all, 2573 samples in 5 years)	25 (42)	no changes in lung function during a work shift; in 5-year longitudinal study larger reduction in FEV_1 in the controls than in the exposed persons	Musk *et al.* 1982
plastic foam production	< 0.05 mg/m^3 (yearly average)	long-term TDI	68 (12)	no changes in spirometric parameters seen in cross-sectional analysis either during a shift or over the 10-year period	Gee and Morgan 1985
plastic foam production	0.05-0.01 mg/m^3 (continuously)	previously TDI	27 (27)	in cross-sectional analysis no significant differences; during a working week significant increase in FEF_{25-75} in exposed persons (on average 14 years)	Sulotto *et al.* 1990

TDI toluylene diisocyanate
VC vinyl chloride/polyvinyl chloride
TCO % CO transfer factor (as % of expected value)
FEF forced expiratory flow
FEV_1 (%) forced expiratory volume in the first second (as % of the expected value)

Another research group compared clinical symptoms and lung function of persons exposed to PMDI during foam insulating procedures during $1^1/_2$ years employment with the same parameters for corresponding control persons (Kolmodin-Hedman *et al.* 1980). The applicable threshold value of 10 ppb (0.1 mg/m^3) PMDI was "attained during short intervals"; peak exposures exceeding the threshold were expressly not excluded and the authors drew attention to the considerable uncertainty associated with the analytical method (modified Marcali method) in the relevant low concentration range. The exposed persons complained more frequently than the controls of workplace-associated symptoms. The average spirometry values of the 18 exposed persons were sometimes slightly lower (not significantly) than those of the 18 control persons. During a work-shift, the FVC increased in the exposed persons by 0.09 litre, the FEV_1 by 0.06 litre. A subsequent examination of 10 of the exposed persons 4 months later revealed no significant changes in lung function.

Another group (Musk *et al.)* carried out lung function tests at the beginning and end of a 5-year period in foam moulding plants where the workers were exposed to mixtures of isocyanates (Musk *et al.* 1982). Originally only TDI was used, then for one year before the second examination PMDI was used as well. The analysis of concentrations in the workplace air was carried out in great detail with 2573 samples in 5 years (20 to 60 minute sampling times; personal samplers, modified Marcali method). The maximum TDI concentration was 15 ppb but 90 % of samples were below 5 ppb. The MDI concentrations were under 4 ppb (0.04 mg/m^3). It was not specified for how long and over which periods the workers had been exposed to isocyanates before the start of the study nor was there any reference to other substances such as amine catalysts to which the workers were exposed. Of the 107 persons re-examined, 25 had been exposed at the end (for 1 year?) only to PMDI. The control group comprised 42 persons. Changes in FEV_1 during a single work-shift were found neither in the control persons nor in those exposed to PMDI. The reduction in FEV_1 over the 5 years was more pronounced in the control group than in the group exposed at the end only to MDI. The quality of the spirometric determinations on which these results were based was later criticized severely (Gee and Morgan 1985). Re-evaluation of the data by the original authors, however, confirmed the original results (Musk *et al.* 1985).

In a study in which 68 workers employed in foam moulding plants were re-examined after 10 years, 42 persons examined in the Musk study in 1982 were included (Gee and Morgan 1985). An additional 12 control persons were examined. The maximum average annual TDI concentration during the period before the study was 3.3 ppb, the maximal MDI concentration 5 ppb (0.05 mg/m^3). During the last two years of the observation period, only PMDI had been used. The spirometry values obtained from the exposed persons before the beginning of the work-shift were slightly better than those from the controls. No significant changes in the spirographic values were observed during the shift. The comparison of the spirometry values from 1971 with those from 1981 proved to be difficult because many of the spirograms from the Musk study were difficult to evaluate (see above). According to the authors' interpretation of the data, no adverse effects on lung function resulted from these isocyanate exposures.

A cross-sectional analysis of lung function parameters was carried out for a small group of persons exposed to low levels of PMDI in a foam moulding plant (Sulotto *et al.* 1990). Until 1980, TDI was used, then increasingly PMDI and, after 1984, only PMDI.

The PMDI concentrations in the workplace air at the time of the lung function tests were between 0.5 and 1.0 ppb (0.005 to 0.01 mg/m^3; continuous tape monitoring). Previous exposure concentrations and the levels of amines at the workplaces are not known. The 27 exposed persons (including 12 smokers) were compared with 27 control persons (including 5 smokers). The two groups of persons had been employed by the company for an average of 14 and 16 years, respectively. The cross-sectional analysis of the spirometric data revealed no significant differences between the two groups. Likewise, there were no differences in the average changes in lung function during and after the shift on Monday and Friday. Comparison of the spirometric values at the beginning and end of the working week revealed only one significant change: an increase of 1.1 in the FEF_{25-75} % [5] in the exposed group.

There are also some other studies which cannot be used in the setting of an occupational exposure limit because of the complete absence of information as to exposure levels (Bertrand *et al.* 1984, Fabbri *et al.* 1976, Hassman 1973, Liss *et al.* 1988, Mapp *et al.* 1976, Mur *et al.* 1982, Pham *et al.* 1982, Saia *et al.* 1976) or because the persons were exposed to poorly defined mixtures (Johnson *et al.* 1985).

Various factors have been suggested to account for the observed deterioration of lung function after exposure to higher isocyanate concentrations: direct irritative effects, cholinesterase inhibition, development of a reactive airway dysfunction syndrome after accidental high-level exposure of individuals in the exposed groups (Luo *et al.* 1990, Roberts 1989).

The attempt to derive a dose-response relationship from the publications cited above (Table 1) is thwarted by great obstacles. Firstly, all the studies have severe weaknesses. The exposure concentrations were mostly incompletely determined and described (sampling time, sampling method and analytical method). The methods used were frequently inappropriate, especially for the determination of PMDI aerosols (simple impingers or tape monitors). In general, the concentrations of PMDI in the workplace air seem to have been underestimated by the early analytical methods (see Note) (Booth 1984, Dharmarajan 1979, Fus 1985, LeBeau 1989, Rosenberg and Pfäffli 1982). Simultaneous exposure to phenyl isocyanate, stripping and expanding agents and amine catalysts was often ignored; particularly exposure to the last-mentioned substance group is likely to be of significance (Belin *et al.* 1983). In general, possible bias in the selection of the collective was not discussed. Allergologic aspects were completely ignored (atopic disposition, time of lung function tests during or outside the pollen season). In all cases, the workers had been exposed to isocyanates for many years before the study and certainly to higher concentrations (Belin *et al.* 1983, Pham *et al.* 1986, 1988); there are no follow-up studies of previously unexposed workers. In all the studies the methods used were dependent on the cooperation of the workers (e.g., spirometry); there are no studies with more objective methods (e.g., whole body plethysmography). The results were usually published in the form of average lung function parameters for the individual collectives; however, detailed information as to how many persons had suffered what degree of lung function impairment would be more useful. Finally, the publication of annual average decreases in spirometric values must be criticized because

[5] FEF forced expiratory flow

the individual decrease depends on the individual initial value and on the phase of the disorder which has been reached (Hoffarth 1991, Postma *et al.* 1979).

With all the uncertainty resulting from the above-mentioned limitations, it may be concluded that significant impairment of lung spirometry values has only been observed in the collective exposed to PMDI concentrations up to 87 ppb (0.9 mg/m^3) (Cavelier *et al.* 1977, Pham *et al.* 1978, 1986, 1988). In collectives exposed to concentrations generally below a threshold value of 20 ppb (0.2 mg/m^3), no significant changes in lung spirometry were found but sometimes significantly more frequent respiratory symptoms. It is not clear whether these were caused by isocyanate exposure or by the simultaneous exposure to other noxious substances. When the PMDI concentrations were less than or equal to 10 ppb (0.1 mg/m^3), such symptoms were no longer significantly more frequent. With the exception of the collective which was known to have been exposed previously to concentrations up to 0.9 mg/m^3, all the collectives exposed to concentrations of 0.05 mg/m^3 or less, sometimes for many years, were without symptoms and had better lung function than the corresponding control collectives.

Almost all studies in which lung function tests were carried out investigated workers in plants producing foam plastics (foam mouldings, insulating foam) where the workers were exposed to mixtures of PMDI, amine catalysts and other additives. Only one study (Diller and Herbert 1982) was carried out with workers in MDI production where the effects of MDI and PMDI could be studied in isolation.

2.3 Specific airway hypersensitivity and sensitization

Airway hypersensitivity to MDI most frequently takes the form of an obstructive disorder of the bronchial airways. On re-exposure, affected persons often first complain of an oppressive feeling, shortness of breath and wheezing. In the classical syndrome, the patients suffer from attacks of respiratory distress ("asthma attacks"). Persistent forms are less frequently seen. The published values for the incidence of MDI asthma are very variable (Bertrand *et al.* 1984, Liss *et al.* 1988, Pezzini *et al.* 1984, Riviera *et al.* 1983, Tanser *et al.* 1973). The figures seem to be affected by a number of factors, particularly the specific diagnostic criteria used and the levels of exposure in the collective involved (Diller 1988, Liss *et al.* 1988). Extensive skin contamination with MDI may also be of relevance: the induction of bronchial hypersensitivity after dermal exposure has been observed in an animal study. The frequency of MDI asthma in large collectives is generally given as 2 % to 14% (Erban 1987, Johnson *et al.* 1985, Lenaerts-Langanke 1992, Martin *et al.* 1982, Seguin *et al.* 1987, Zammit-Tabona *et al.* 1983). In collectives exposed to PMDI concentrations of less than 5 ppb (0.05 mg/m^3), the incidence of asthma attacks or asthmatic symptoms was not increased (Gee and Morgan 1985, Musk *et al.* 1982, Pham *et al.* 1986, 1988).

MDI asthma is generally associated with non-specific bronchial hyper-responsiveness. An increase in the incidence of nonspecific bronchial hyper-responsiveness as a symptom on its own has been observed after exposure to high levels of PMDI mixed with aliphatic tertiary amine catalysts (Johnson *et al.* 1985), as after the corresponding mixed exposures with TDI (Belin *et al.* 1983).

An exposure-induced cough has been described repeatedly as an early form of bronchial hypersensitivity (Alt and Diller 1988, Belin 1982). Once airway hypersensitivity has developed with its associated symptoms, a single re-exposure to a PMDI concentration of 0.09 to 0.1 mg/m^3 for one hour can lead to an increase in airway resistance (Fruhman *et al.* 1987).

Immunological changes have been detected in some persons with PMDI hypersensitivity. Mostly, the level of specific IgE antibodies was increased (Baur *et al.* 1984, Chang and Karol 1984, Liss *et al.* 1988, Tse *et al.* 1985, Wass and Belin 1989, Zammit-Tabona *et al.* 1983, Zeiss *et al.* 1980). Less frequently, an increase in specific IgG was seen (Chang and Karol 1984, Liss *et al.* 1988, Tse *et al.* 1983, 1985, Zammit-Tabona *et al.* 1983, Zeiss *et al.* 1980) or a whole spectrum of polyclonal antibodies (Chang and Karol 1984, Chen and Bernstein 1982). However, specific antibodies have repeatedly been found in asymptomatic exposed persons, perhaps as expression of latent sensitization or sensitization without organic manifestation (Butcher *et al.* 1983, Liss *et al.* 1988). The difficulties associated with the determination of such immunological parameters have often been emphasized (Baur 1987, Karol and Thome 1988, Wass and Belin 1989).

An early publication (Konzen *et al.* 1966) described antibody formation in workers exposed to peak PMDI concentrations of more than 1.51 mg/m^3 (4 minutes) but not after similar exposures to 1.35 mg/m^3. The methods used for air analysis and the immunological methods were, however, as we now know, unreliable so that the validity of these results must be considered doubtful.

Not only humoral immunological mechanisms have been suggested to be operative in specific bronchial hypersensitivity caused by isocyanates but also cellular immunological processes involving lymphocyte sub-populations and pharmacological mechanisms such as inhibition of cholinesterase and adenylate cyclase.

A few cases of so-called exogenic allergic alveolitis (hypersensitivity pneumoni-tis) have been described in persons exposed to PMDI. The classical syndrome is characterized by influenza-like symptoms such as fatigue, malaise and arthralgia, raised temperature, coughing, changes in blood count and in the bronchio-alveolar lavage fluid and shadows in the pulmonary radiograph (Bahemann-Hoffmeister *et al.* 1989, Bascom *et al.* 1985, Baur and Fruhmann 1983, Beysens *et al.* 1985, Brochard *et al.* 1982, Friedman 1982, Garde *et al.* 1989, Lob 1972, Lob and Boillat 1981, Malo and Zeiss 1982, Malo *et al.* 1983, Schwarz *et al.* 1989, Vergnon *et al.* 1985, Walker *et al.* 1989, Zeiss *et al.* 1980, Zschiesche 1991). Occasionally, the symptoms of alveolitis were associated with asthmatic symptoms (Baur and Fruhmann 1983, Malo *et al.* 1983). An associated increase in specific IgG has also been described (Walker *et al.* 1989). In one case, pulmonary fibrosis developed (Friedman 1982).

2.4 Effects on the skin and other organs

On the skin, MDI and PMDI can have a transient tanning effect with dark discoloration and occasionally cause erythema as well. Allergic contact dermatitis caused by MDI is extremely rare and appears to develop only under conditions of poor occupational hygiene (Fregert 1967, Lidén 1980, Mas and Heinz 1984, Moroni *et al.* 1985, Rothe 1976).

3 Effects of monomeric MDI on Animals

3.1 Inhalation studies with single or repeated exposure

During a single 3-hour exposure to concentrations of monomeric MDI aerosol between 0.6 and 350 mg/m^3 (no other details), the guinea pigs of the low concentration groups reacted with a decrease in respiration rate and an increase in tidal volume, whereas above a concentration of 10.4 mg/m^3 a concentration-dependent increase in respiration rate was seen (Thorne *et al.* 1986 a). During 3-hour exposure to a monomeric MDI concentration of 78.4 mg/m^3, the respiration rate of guinea pigs first increased and then decreased during the exposure to about 36 % of the control value. At the same time the body temperature sank by 3 °C. Whereas the respiration rate had not returned to the initial value by 24 hours after the end of exposure, the body temperature had returned to normal at this time (Thorne *et al.* 1987 b).

In contrast, in mice exposed to MDI aerosol concentrations between 10.2 and 58.5 mg/m^3 (no other details) for 4 hours, the respiration rate was decreased in a manner dependent on concentration and exposure duration (RD_{50} 32 mg/m^3) [6], accompanied by a concentration-dependent increase in lung weights (Weyel and Schaffer 1985).

More recent studies with repeated exposures have not been described.

3.2 Sensitizing Effects

Pulmonary sensitization was achieved by exposing guinea pigs to monomeric MDI aerosol concentrations of 17.4 mg/m^3, 3 hours daily for 5 days for induction and subsequent provocation by exposure to 2.5 mg/m^3 (Thorne *et al.* 1986 a), and also by induction with 22.4 mg/m^3, 3 hours daily for 5 days, followed by provocation after 3 and 5 weeks by exposure to 3 –10 mg/m^3 for 1 hour (Griffiths-Johnson *et al.* 1990). In both studies the treated guinea pigs reacted after a latent period with a reduction in tidal volume and simultaneous increase in respiration rate which had decreased to 70% of the control value after the induction exposure with the monomeric MDI concentration of 22.4 mg/m^3. After inhalation sensitization of guinea pigs, a positive skin reaction could be produced with a subsequent intrader-mal injection of an MDI-globulin conjugate (Thorne *et al.* 1986 a). The reverse process was also possible: after intradermal injection of monomeric MDI, specific IgE antibodies were detected in the blood (Jin and Karol 1988) and pulmonary hypersensitivity reactions could also be demonstrated (Botham 1990).

With the maximization test of Magnusson and Kligman, severe contact allergies with cross reactions with TDI and the corresponding amines were produced in guinea pigs treated with a mixture of monomeric MDI (main component) and other diisocyanates and triisocyanates (minor components) (Duprat *et al.* 1976).

[6] RD_{50} concentration causing a 50 % decrease in respiration rate

When monomeric MDI was tested for sensitization in the mouse ear swelling test (MEST), the response was only weakly dose-related in the dose range between 0.4 and 0.6 mg/kg body weight; between 0.6 and 37 mg/kg body weight, however, there was a marked dose-response relationship; the reaction achieved with 187 mg/kg body weight was only 55 % of the maximal response (Thome *et al.* 1986 b, 1987 a). It could also be shown with the MEST after application of a 1 % solution of monomeric MDI in ethyl acetate to the mouse ear that the treated ears swell after a latent period and that the swelling reaches a maximum 24 hours after provocation (Tanaka *et al.* 1987). Cross-reactions between the isocyanates were also demonstrated with the MEST (Tanaka *et al.* 1987, Thorne *et al.* 1986 b, 1987 a).

In another study, lymphocytes were obtained from lymph nodes or from the spleen of mice which had previously been sensitized by dermal treatment with monomeric MDI and were injected into the tail veins of untreated mice (Tanaka *et al.* 1987). The subsequent dermal provocation of the latter mice with monomeric MDI led to swelling of the test ears only in mice which had been treated with lymph node lymphocytes. Since treatment of the animals with monoclonal anti-Thy-1,2-antibodies and complement before the provocation prevented the reaction, the authors concluded that it involved T cell-mediated contact sensitivity.

3.3 Reproductive and Developmental Toxicity

The effect of monomeric MDI on reproduction has not yet been investigated.

3.4 Genotoxicity

The data available from genotoxicity studies with monomeric MDI are shown in Table 2.

In most of the Ames tests which have been carried out to date, an increase in the number of revertants was found in the two *Salmonella typhimurium* strains TA98 and TA100 only in the presence of S9 mix; the solvents used were dimethyl sulfoxide (DMSO) and acetone. When MDI was dissolved in ethylene glycol dimethyl ether (EGDE), no mutagenic activity could be demonstrated in TA100 (Herbold 1990 b).

In none of the studies was the stability of the test substance in the solvent investigated. This proved to be a mistake because later studies provided evidence that the differences in the results could originate in solvent effects. In addition, it must be taken into account that *in vitro,* MDI reacts in aqueous medium to form polyurea and 4,4'-diaminodiphenylmethane (DDPM), which in turn reacts with MDI. In highly acid medium the substance is practically completely hydrolysed (Diller 1981).

Table 2. Genotoxicity studies with monomeric MDI

Test system	Indicator organism	Dose or concentration (exposure period)	Solvent	S9mix	Test result	References
Gene mutation tests in bacteria						
Ames test	*S. typhimurium*	in each case				Herbold 1980 a
	TA98, TA100, TA1535, TA1537	4 – 2500 µg/plate	DMSO	none	–	
	TA98, TA100			with rat liver	+	
	TA1535, TA1537			with rat liver	–	
Ames test	*S. typhimurium* TA100	4 – 2500 µg/plate	acetone	with rat liver	+	Herbold 1980 b
Ames test	*S. typhimurium*	in each case				Andersen *et al.* 1980
	TA98, TA100, TA1537	max. 100 µg/plate	DMSO	none	–	
	TA98, TA100			with rat liver	+	
	TA1537			with rat liver	–	
Ames test	*S. typhimurium*	in each case	n.s.			Haskell Laboratories 1976
	TA98, TA100, TA1535, TA1537, TA1538	max. 2500 µg/plate		none	–	
	TA98, TA100			with rat liver	+	
	TA1535, TA1537, TA1538			with rat liver	–	
Ames test	*S. typhimurium*					Reichold Chemicals 1982
	TA98, TA100, TA1535, TA1537, TA1538	n.s.	n.s.	none	–	
				with rat liver	–	
Ames test	*S. typhimurium* (strains n.s.)	n.s.	n.s.	with rat liver	+	Foderaro 1978
Ames test (with preincubation, 20 min)	*S. typhimurium*	in each case				Shimizu *et al.* 1985
	TA98, TA100, TA1535, TA1537, TA1538	5 – 5000 µg/plate	DMSO	none	–	
	TA98, TA 100			with rat liver	+	

Table 2: (continued)

Test system	Indicator organism	Dose or concentration (exposure period)	Solvent	S9mix	Test result	References
Ames test (with preincubation, 20 min)	TA1535, TA1537, TA1538 E. coli WP2uvrA E. coli WP2uvrA	in each case 5 – 5000 µg/plate	DMSO	with rat liver none with rat liver	– – –	Shimizu *et al.* 1985
Ames test (with preiecubation, 20 min)	*S. typhimurium* TA98, TA100, TA1535, TA1537	in each case 10 – 10000 µg/plate	DMSO	none with rat liver with hamster liver	– – –	Zeiger *et al.* 1987
Ames test	*S. typhimurium* TA98 *E. coli* WP2uvrA	in each case 1.56 – 6.25 ml urine*	DMSO	with rat liver with rat liver	(?) (?)	Holmén *et al.* 1988
Ames test	*S. typhimurium* TA100	150 – 2400 µg/plate	EGDE	none with rat liver	– –	Herbold 1990 b
Tests for point mutations in mammalian cells *in vitro*						
TK test	L5178Y mouse lymphoma cells	2.5 – 250 µg/ml (3h)	DMSO	none with rat liver	– + **	McGregor *et al.* 1981 a
Tests for chromosomal aberrations *in vitro*						
chromosomal aberration test	human lymphocytes	0.54 – 4.3 µg/ml*** (24 h) (1.5h)	acetone	none with rat liver	+ +	Mäki-Paakkanen and Norppa 1987
sister chromatid exchange	human lymphocytes	0.12 – 5.4 µg/ml*** (48 h) (1.5h)	acetone	none with rat liver	(+) (+)	Mäki-Paakkanen and Norppa 1987

Table 2: (continued)

Test system	Indicator organism	Dose or concentration (exposure period)	Solvent	S9mix	Test result	References
Tests for chromosomal aberrations *in vivo*						
chromosomal aberration test	human lymphocytes from workers exposed to MDI among other substances		culture medium RPMI 1640		–	Holmén *et al.* 1988
sister chromatid exchange	human lymphocytes from workers exposed to MDI among other substances		culture medium RPMI 1640		–	Holmén *et al.* 1988
micronucleus test	human lymphocytes from workers exposed to MDI among other substances		culture medium RPMI 1640		–	Holmén *et al* 1988
micronucleus test	mouse	32, 80 or 200 mg/kg bw i.p.	DMSO		–	JETOC 1982
Test for cell transformation *in vitro*						
cell trans-formation test	BHK 21 C13 cells	125 – 2000 µg/rnl (4h)	DMSO	none with rat liver	+ +	Poole and Harris 1980 a

n.s.	not specified
DMSO	dimethyl sulfoxide
EGDE	ethylene glycol dimethyl ether
RPMI 1640	Roswell Park Memorial Institute cell culture medium 1640
*	urine samples from workers exposed to MDI among other substances
**	only at 250 µg/ml; cytotoxic concentration
***	technical product with 45 % MDI
(?)	number of revertants not significantly increased
(+)	weak positive
BHK 21 C13 cells	Syrian hamster kidney cells
bw	body weight

It is not known whether DDPM is formed under the conditions of the Salmonella/ microsome test after addition of the MDI to the aqueous phase. DDPM itself yields positive results in the Ames test in *S. typhimurium* TA98 and TA100 in the presence of S9 mix (see 4,4'-Diaminodiphenylmethane, Volume 7 of this series). With MDI dissolved in EGDE (water content 0.11 %), negative results were obtained in the Ames test. Under these conditions, therefore, only traces of DDPM can have been formed.

To investigate the question of whether the solvent affects the result of the mutagenicity test, the stability of MDI in EGDE (negative result) and in DMSO (positive result) were compared. MDI is stable in EGDE (Fus and Katzenstein 1991). In contrast, HPLC analysis demonstrated that MDI is not stable in DMSO (water content 0.04%) (Fus and Katzenstein 1991). Freshly prepared solutions have only 22.1 % of the initial MDI content after as little as 15 minutes, after 30 minutes the MDI level has dropped to 1 % and after 45 minutes no free MDI can be detected. The batches of DMSO used had a lower water content (0.04 %) than did the EGDE employed in the study described above (0.11 %). The formation of mutagenic products after dissolution of the substance in DMSO is therefore not a result of reaction with water but probably involves a still unknown DMSO-dependent mechanism. The conditions necessary for the formation of mutagenic products from MDI in DMSO are not known at present. Therefore it is not possible to account for the one negative result for MDI in *S. typhimurium* TA98 and TA100 in the Salmonella/ microsome test with DMSO as solvent (Zeiger *et al.* 1987) nor for the negative result of one micronucleus test (JETOC 1982). The positive result of a cell transformation test *in vitro* in which the MDI was dissolved in DMSO could also be ascribed to the DMSO-dependent formation of mutagenic substances (Poole and Harris 1980 a).

Positive results were obtained in a test for chromosomal aberrations *in vitro* with MDI in an aqueous acetone solution (Mäki-Paakkanen and Norppa 1987). However, the stability of MDI in this solvent was not investigated, neither for this study nor for a Salmonella/microsome test which also yielded positive results (Herbold 1980 b).

Negative results were obtained in a study of the clastogenic activity of MDI in the lymphocytes of workers exposed to MDI among other substances (Holmén *et al.* 1988). On the other hand, DNA damage was reported in the genome of a worker who had inhaled MDI (Marcynski *et al.* 1992). The methods applied in this study have not been validated nor described in the literature. A final assessment of these findings can, therefore, not yet be carried out.

3.5 Carcinogenicity

There are no carcinogenicity studies with monomeric MDI.

3.6 Other effects

In *in vitro* studies with monomeric MDI, the substance was shown to inhibit acetyl cholinesterase and adenylate cyclase (Dewair *et al.* 1983, McKay *et al.* 1981).

4 Effects of polymeric MDI (PMDI) on animals

The studies which are described below were carried out with a PMDI test sample with an NCO content specified as 30 ± 2 % w/w and a monomeric MDI level of 52 ± 3% w/w.

4.1 Inhalation studies with single or repeated exposure

In a short-term inhalation study (Appelman and de Jong 1982 a), groups of 5 SPF-Wistar rats per sex and concentration were exposed for 4 hours to determine the LC_{50} and further groups of 5 SPF-Wistar rats per sex and concentration for the pathological examination; the average exposure concentrations were 523, 500, 418 and 384 mg/m^3 of a PMDI aerosol in which 99.6 % to 99.7 % of particles were smaller than 5 µm. The particle size distribution and the concentrations were determined either with a Cascade impactor or G4 filter and by spectrophotometry with the oil-red method or by high pressure liquid chromatography (HPLC). During the exposure the animals sat quietly with closed eyes and, especially in the highest concentration group, their breathing became laboured and nostrils dilated. At the end of the experiment, the dorsal fur of the animals was discoloured pale pink (oil-red). Autopsy of the animals intended for the pathological examination was carried out immediately after the end of exposure. Haemorrhage and oedema were found in the lungs and, in the animals of the highest concentration group the lungs were also discoloured grey. Of the rats exposed for the LC_{50} determination, 2 females and 3 males of the 523 mg/m^3 group, 4 females and 2 males of the 500 mg/m^3 group,

3 females and 1 male of the 418 mg/m^3 group and 1 female of the 384 mg/m^3 group died during the exposure or the subsequent 2-week recovery period. The LC_{50} was found to be 490 mg/m^3 when the concentrations were determined with the oil-red method and 456 mg/m^3 by HPLC.

In another inhalation study (Appelman and de Jong 1982 b) with whole-body exposure, 5 male Wistar rats per concentration group were exposed to a PMDI aerosol concentration which was determined as 31.3 mg/m^3 spectrophotometrically and 25.5 mg/m^3 by HPLC. The animals were exposed once for 6 hours or 6 hours daily for 3 or 5 days. Determination of the diameter of the aerosol particles on days 0, 2 and 4 with a 10-stage Berkeley quartz crystal balance (QCM) cascade impactor demonstrated that 95.3 % to 97.6 % of the particles were smaller than 5 µm. To investigate whether the aerosol was deposited on the fur of the animals and whether DDPM was formed, the animals were shaved after the exposure on days 0, 2 and

4 and the hair collected and extracted with toluene. HPLC analysis of the toluene extract revealed monomeric MDI levels of 12 to 34 µg/g hair (16 to 50 µg/animal) but no DDPM. The described clinical effects included laboured breathing which developed after the second exposure and became more marked after further exposures. After 5 exposures, the animals were in a poor general state. The average body weight, measured in a group of 5 animals after 4 exposures, was reduced by 30 %.

In a 2-week inhalation study, male and female rats were exposed to PMDI aerosol concentrations of 0, 2.18, 4.88 or 13.55 mg/m^3 (values from determinations by HPLC and by the oil-red method averaged), 6 hours daily on 5 days per week.

Analysis of 10 samples per concentration with a QCM cascade impactor revealed that the diameters of 97.1 % to 100 % of the aerosol particles were less than 5 µm. In the animals in the 4.88 mg/m^3 group, respiratory distress and delayed body weight gain developed whereas at the highest concentration severe respiratory insufficiency led to death of 7/10 male and 1/10 female rats. The authors stated that in this test series, the lowest PMDI concentration producing an effect was 2.18 mg/m^3. HPLC analysis revealed no DDPM in the atmosphere (Reuzel 1985 a).

Further studies of 4-week and 6-week old rats exposed under the same conditions to the same concentrations as those above confirmed the finding of high mortality in the highest concentration group and demonstrated that younger rats appear to be more sensitive to the toxic effects of PMDI than are older animals (Reuzel 1985b).

Groups of 15 male and 15 female rats were exposed to PMDI aerosol (95 % < 5 µm) concentrations between 0 and 5.03 mg/m^3, 6 hours daily, 5 days per week for 13 weeks. The only apparent effect was a slight delay in growth in the highest concentration group. Histological examination of liver, kidneys and respiratory tract revealed yellow deposits in the respiratory tract only in the group exposed to the highest concentration (Reuzel *et al.* 1985). The authors concluded from these results that no clear adverse effect level could be identified with the concentrations used. Therefore, in a subsequent study (Reuzel *et al.* 1986 b), groups of 30 rats of each sex were exposed under the same conditions to PMDI concentrations of 0, 4.06, 8.43 and 12.25 mg/m^3 (measured by gravimetry, photometry and HPLC; methods described in Reuzel *et al.* 1985). The concentrations were also monitored continuously with a Simslin aerosol light scattering photometer. A Berkeley QCM cascade impactor showed the diameter of more than 95 % of the aerosol particles to be less than 5 µm (no other details). In the 8.43 mg/m^3 group, the animals developed dyspnoea and their body weight gain was slightly reduced; the animals of the highest concentration group developed severe respiratory insufficiency accompanied by significant reduction in body weight gain with subsequent death of 11/30 males and 4/30 females. Whereas the opthalmological and haematological examinations yielded no pathological findings in any of the animals, determination of blood chemical parameters revealed increased creatinine values in the females of the two highest concentration groups. In these two groups the lung weights were also increased; the gross pathological examination revealed no adverse effects in this group or in the others. The histopathological examination, however, revealed in all concentration groups degenerative and hyperplastic lesions of the respiratory and olfactory epithe-lia of the nasal cavity, aggregations of macrophages with reduced phagocytosis activity and yellowish inclusions as well as concentration-dependent inflammatory reactions in the pulmonary tissue. Macrophage aggregations were also detected in the mediastinal lymph nodes but without inflammatory tissue changes. At the end of the 4-week recovery period there was clear evidence that regeneration had begun. The authors concluded from these results that the two highest concentrations caused marked damage; even the lowest concentration of 4.06 mg/m^3 still caused slight changes.

In week 11 of the 13-week study, traces of DDPM (15 μm/m^3) were detected in all test atmospheres and in the control atmosphere. An additional 2-day aerosol study was carried out under the same conditions with 0.2 and 5.0 mg/m^3 PMDI to establish whether the DDPM originated as an impurity or from hydrolysis of MDI (Appelman *et al.* 1986). The aerosol samples were collected with a modified im-pinger, a U-tube collector with a large diameter and with two solvents, toluene and dichloromethane. In none of the 32 samples was DDPM found. Neither was DDPM detectable with the impinger method and dichloromethane as solvent. The authors concluded that the detected DDPM was an artefact. Isocyanates can react with water in various ways and, depending on the ionic strength, temperature, pH, concentration of reactants (including solvent, solubilizers and biological macro-molecules), competing reactions can result in the formation of amines, isocyanate-ureas, amine-ureas or polyureas (Brown *et al.* 1991, Saunders and Frisch 1962). In acid stomach juices, for example, the hydrolysis of TDI to TDA takes precedence over the reaction with biological macromolecules whereas under neutral conditions the reaction of TDI with functional groups of the macromolecules predominates (Brown *et al.* 1991). Because MDI and its reaction products are poorly soluble, the possible reactions of MDI are more limited than those of TDI.

In a long-term inhalation study (Reuzel *et al.* 1990), groups of 60 male and 60 female Wistar rats (main group) were exposed to PMDI aerosols, 6 hours daily, 5 days per week for 2 years and further groups of 10 male and 10 female rats (satellite group) for 1 year. The aerosol was produced with a nebulizer developed by CIVO TNO. The target PMDI concentrations were 0.2, 1.0 and 6.0 mg/m^3, since no excessive mortality was expected at the highest concentration and the lowest was thought to be the no toxic effect level. Gravimetry, β-attenuation and HPLC analysis were chosen as analytical methods suitable for determining the aerosol concentrations. A Simslin aerosol light scattering photometer was used to monitor the flow (Reuzel *et al.* 1986a). The average concentrations determined were 0.19 ± 0.05, 0.98 ± 0.11 and 6.03 ± 0.54 mg/m^3 for which the aerosol particles had median aerodynamic diameters of 0.68 ± 2.93, 0.70 ± 2.46 and 0.74 ± 2.31, respectively, as determined at weekly intervals with a 10-stage Berkeley quartz crystal balance (QCM) cascade impactor (c.f. 13-week study).

In the **satellite groups** after one year, the only finding in the animals exposed to 0.2 mg/m3 was a significantly reduced Na level in the blood plasma of male animals. There were no other significant differences between the animals of this group and the controls. The Na level in plasma was also significantly reduced in the male animals of the 1.0 mg/m3 group. In the females of this group, the haematocrit was significantly reduced. There were no gross pathological differences between the exposed and control animals. Histopathological examination revealed basal cell hyperplasia of the olfactory epithelium in the nasal cavity in some of the animals and in some aggregations of macrophages loaded with yellowish material in the alveolar passages of the subpleural region. In the area around these macrophage aggregations, the flat alveolar epithelium had been converted to cubic epithelium and the number of connective tissue fibres was increased. In addition, yellow coloured macrophages were found frequently in the mediastinal lymph nodes. In the male rats of the 6.0 mg/m^3 group, the blood Na level was slightly decreased. There were no differences in gross pathology between the exposed animals and the controls. However, the relative lung weights (expressed in

terms of the body weights) of these animals were increased significantly relative to the control values. Histopathological examination revealed significant changes in the nasal cavity, in the lungs and in the mediastinal lymph nodes. There was an increased incidence of basal cell hyperplasia and slight damage to the olfactory epithelium in the nasal cavity in males of this group (no other details). Aggregations of macrophages loaded with yellowish pigment were found in the alveolar passages of the subpleural region in the lungs of all animals in this group. In the area around these aggregates, the flat alveolar epithelium had been converted to a cubic epithelium and the number of connective tissue fibres was increased. In addition, aggregates of yellow coloured macrophages were found in the mediastinal lymph nodes of most animals in this group.

In the **main study,** autopsy revealed increased lung weights only in the animals of the highest concentration group; they were accompanied by increased mottling of the pulmonary surface and/or increased paleness of the tissue. Microscopic changes seen in the pathological examination were like those already seen in the satellite groups: changes in the nasal cavity, the lungs and the mediastinal lymph nodes. Basal cell hyperplasia was observed in the olfactory epithelium, often associated with epithelial atrophy and hyperplasia of Bowman's glands. In most animals of this group, aggregations of yellowish coloured macrophages were seen in the lumen of the alveoli with epithelialization of the alveolar passages and local fibrosis. The macrophage aggregations, especially in the females, were often accompanied by occasional roundish calcareous deposits in the alveoli and bronchi or alveolar interstitium. In addition, in the animals of the 6.0 mg/m^3 group, bronchialization of the alveoli was frequent and characterized by conversion of the flat alveolar epithelium into an epithelium constructed of cubic or columnar cells like the cells of the bronchial epithelium but retaining the subepithelial structure. In most animals of this group, there were aggregations of yellowish coloured macrophages in the mediastinal lymph nodes. The same changes occurred in a less pronounced form in only a few animals of the 1.0 mg/m^3 group. In the 0.2 mg/m^3 group, no differences from the control group could be found. Microscopic changes seen in the other organs examined were typical for rats of this age and strain and did not represent treatment-related effects. In addition, these changes occurred in the exposed and control groups with equal frequency and severity and were not dose-dependent. Whereas the tumour incidences in the low and medium dose groups were not increased, and especially no lung tumours were found, in the 6.0 mg/m^3 group, lung adenomas were found in 6 males and 2 females and one adenocarcinoma in 1 male (Section 4.5).

4.2 Sensitization

The maximization test of Magnusson and Kligman yielded negative results with PMDI in guinea pigs (Schmidt and Bombard 1984). After intradermal injection of PMDI, specific antibodies of IgE type were detected in the blood (Chang and Karol 1984).

4.3 Reproductive and developmental toxicity

The effects of PMDI on reproduction have not yet been investigated.

4.4 Mutagenicity

The available mutagenicity data for PMDI are shown in Table 3. In the two Ames tests with PMDI in which DMSO was used as the solvent, increased numbers of revertants were found only in strain TA100 in the presence of S9 mix. When EGDE was used as the solvent, as with monomeric MDI, no mutagenic activity could be detected. These results suggest that in the case of PMDI too, DMSO could be causally involved in the mutagenic activity. That could also be the reason why PMDI, like monomeric MDI, produced an increase in transformation rate in the cell transformation test when DMSO was the solvent. The test for point mutations in mammalian cells with DMSO as solvent yielded negative results with PMDI, unlike with monomeric MDI. However, the results are not strictly comparable because toxic concentrations at which the mutagenic effect became apparent were tested only for monomeric MDI.

4.5 Carcinogenicity

In the long-term inhalation study described above in which rats inhaled average PMDI concentrations of 0.19, 0.98 or 6.03 mg/m^3, 6 hours daily, 5 days per week for 2 years, tumours were found only in the highest dose group, lung adenomas in 6 males and 2 females and an adenocarcinoma in 1 male (Reuzel *et al.* 1990). The tumour aetiology was deduced from the results of detailed histopathological examinations. The process appears to be triggered by the cytotoxic effects of PMDI, especially for the type I pneumocytes which line the centroacinar regions of the lungs. Alveolar macrophages phagocytose the foreign material. Local destruction of the epithelium exposes the basal membrane or damages the interstitium. Then repair of the interstitium (by fibroblasts) and epithelium begins. At the same time, the phagocytosis of test substance by activated macrophages results in production of growth factors for both fibroblasts and epithelial cells, especially for type II pneumocytes. As a result, fibroblasts and epithelial cells proliferate. Possibly mediated by another factor produced by the macrophages, the formation of hyperplastic type II pneumocytes continues and accelerates until a small number of lung adenomas is formed in the area of the most pronounced type II pneumocyte hyperplasia and macrophage aggregation. The authors also propose that the progression to solid adenomas and/or carcinomas may be caused both by the continuous proliferation of the type II cells and by an increasing mutation rate (Reuzel *et al.* 1994). Whether or not this involves genotoxic action of PMDI or its mutagenic hydrolysis product DDPM (see 4,4'-Diaminodiphenylmethane, Volume 7 of this series) cannot be assessed at present.

Table 3. Genotoxicity studies with "polymeric MDI" (PMDI)

Test system	Indicator organism	Dose or concentration (exposure period)	Solvent	S9mix	Test result	References
Tests for gene mutations in bacteria						
Ames test	*S. typhimurium* TA100	max. 500 µg/plate	DMSO	none	–	Andersen *et al.* 1980
				with rat liver	+	
Ames test	*S. typhimurium* TA98, TA100, TA1535, TA1537	in each case 20 – 2500 µg/plate	DMSO			Herbold 1980c
	TA100			none	–	
				with rat liver	+	
	TA98, TA1535, TA1537			with rat liver	–	
Ames test	*S. typhimurium* TA100	150 – 2400 µg/plate	EGDE	none	–	Herbold 1990 a
				with rat liver	–	
Ames test	*S. typhimurium* TA1535, TA1537 TA1538	n.s.	n.s.	none	–	Haskell Laboratories 1975
				with rat liver	–	
Test for point mutation in mammalian cells *in vitro*						
TK test	L5178Y mouse lymphoma cells	2.5 – 250 µg/ml (3h)	DMSO	none	–	McGregor *et al.* 1981 b
				with rat liver	–	
Test for cell transformation *in vitro*						
Cell transform-mation test	BHK 21 Cl 3 cells	125 – 2000 µg/ml (4h)	DMSO	none	+	Poole and Harris 1980 b
				with rat liver	+	

For explanations see footnote to Table 2
n.s. not specified

5 Manifesto (MAK value, classification)

Both in animal studies and in man, the predominant effects of 4,4'-methylene diphenyl isocyanate (MDI) are irritation and sensitization of the respiratory tract. High concentrations cause reduced respiration rate, dyspnoea and respiratory insufficiency.

In studies of occupational health, significant (reversible) adverse effects on lung function were observed in persons exposed to MDI concentrations above a threshold of 0.2 mg/m^3. Provided that MDI concentrations were kept largely below this threshold, significant changes in lung spirometry were no longer seen but the incidence of respiratory symptoms was increased significantly. If MDI concentrations were generally kept below a threshold of 0.1 mg/m^3, such disorders were still observed but no more frequently than in the control group. If MDI concentrations were kept largely below a threshold of 0.05 mg/m^3, there was no increase in the incidence of respiratory symptoms and the lung function was normal before and after the work-shift; the lung function parameters remained in the normal range during 10 years of such exposures.

Because of these observations, the MAK value for MDI was reduced in 1992 to 0.005 ml/m^3, that is, 0.05 mg/m^3.

For the induction of specific airway hypersensitivity (with or without immunological parameters), exposure to MDI concentrations above 0.2 mg/m^3 or intensive skin contact seem to be important. Long-term exposure to MDI concentrations of 0.05 mg/m^3 or less causes neither bronchial hypersensitivity with its associated symptoms nor the formation of specific antibodies.

Intensive skin contact with MDI causes irritation. Although dermal sensitization can be produced in animal studies, cases of allergic contact dermatitis caused by MDI are rarely seen by occupational physicians. In animal studies, intradermal injection of MDI causes bronchial sensitization.

The sensitizing potential of MDI is only moderate in comparison with that of other sensitizing substances encountered at the workplace. Differences in the effects of monomeric MDI and "polymeric MDI" (PMDI) on human lung function parameters and bronchial sensitization have not been established. In animal studies, the production of contact eczema on the skin has been achieved only with monomeric MDI.

Since the results obtained with MDI and PMDI in mutagenicity tests vary with the solvent used, it is not yet possible to assess the genotoxic potential of these substances. Further studies are therefore necessary.

In a long-term inhalation study, exposure of rats to PMDI caused chronic inflammatory changes in the respiratory tract and an increased incidence of lung tumours. These tumours developed at exposure concentrations which produce persistent inflammatory changes in the respiratory tract. Because of these findings, inspirable aerosols of MDI and PMDI are classified in category IIIB in the "List of MAK and BAT Values". The results of an inhalation study with monomeric MDI which is still in progress must be awaited before the carcinogenic potential of the substance can be assessed.

Because of its sensitizing potential, MDI is designated with an "S". A local irritant, MDI is classified in category I for the limitation of short-term exposure peaks (peak level twice the MAK value, duration not more than 5 minutes, frequency up to 8 times per shift).

Since there are no studies of the reproductive toxicology of MDI, the substance cannot be classified in one of the pregnancy risk groups and is included in Section IIc of the "List of MAK and BAT Values".

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