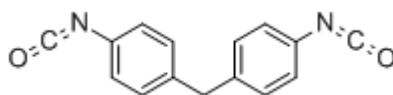


# Methylenediphenyl diisocyanate

(Methylenebis(4-phenylisocyanate), diphenylmethane diisocyanate, 1,1'-Methylenebis(4-isocyanatobenzene), MDI)

CAS: 101-68-8



## 1. Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). OEHHA developed a Technical Support Document (TSD) in response to this statutory requirement that describes acute, 8 hour and chronic RELs and was adopted in December 2008. The TSD presents methodology reflecting the latest scientific knowledge and techniques, and in particular explicitly includes consideration of possible differential effects on the health of infants, children and other sensitive subpopulations, in accordance with the mandate of the Children's Environmental Health Protection Act (Senate Bill 25, Escutia, chapter 731, statutes of 1999, Health and Safety Code Sections 39669.5 *et seq.*). These guidelines have been used to develop the following RELs for methylene diphenyl diisocyanate: this document will be added to Appendix D of the TSD.

Exposure to diisocyanates including methylene diphenyl diisocyanate has been found to cause adverse effects on the respiratory system in both animals and humans. These effects include acute impacts such as sensory irritation and the induction of asthma in sensitive subjects. There are also chronic effects such as long-term decrements in lung function and sensitization, resulting in the induction of asthma and triggering of attacks following even very low exposures to diisocyanates.

### 1.1 Methylenediphenyl diisocyanate Acute REL

<i>Reference exposure level</i>	5 µg/m <sup>3</sup> (0.49 ppb)
<i>Critical effect(s)</i>	Fetal anomalies
<i>Hazard index target(s)</i>	Skeleton, viscera

### 1.2 Methylenediphenyl diisocyanate 8-hour REL

<i>Reference exposure level</i>	0.05 µg/m <sup>3</sup> (0.005 ppb)
<i>Critical effect(s)</i>	Hyperplasia of olfactory epithelium
<i>Hazard index target(s)</i>	Upper respiratory tract

### 1.3 Methylenediphenyl diisocyanate Chronic REL

<i>Reference exposure level</i>	0.05 µg/m <sup>3</sup> (0.005 ppb)
<i>Critical effect(s)</i>	Hyperplasia of olfactory epithelium
<i>Hazard index target(s)</i>	Upper respiratory tract

## 2. Physical & Chemical Properties (HSDB 2004)

<i>Description</i>	Light yellow, fused solid (23°C)
<i>Molecular formula</i>	C <sub>15</sub> H <sub>10</sub> N <sub>2</sub> O <sub>2</sub>
<i>Molecular weight</i>	250.25 g/mol
<i>Density</i>	1.23 g/cm <sup>3</sup> (25°C)
<i>Boiling point</i>	196°C
<i>Melting point</i>	37°C
<i>Vapor pressure</i>	5 x 10 <sup>-6</sup> mm Hg @ 25°C
<i>Odor threshold</i>	odorless
<i>Solubility</i>	Acetone, benzene, kerosene, nitrobenzene.
<i>Conversion factor</i>	10.24 mg/m <sup>3</sup> = 1 ppm @ 25° C

## 3. Major Uses and Sources

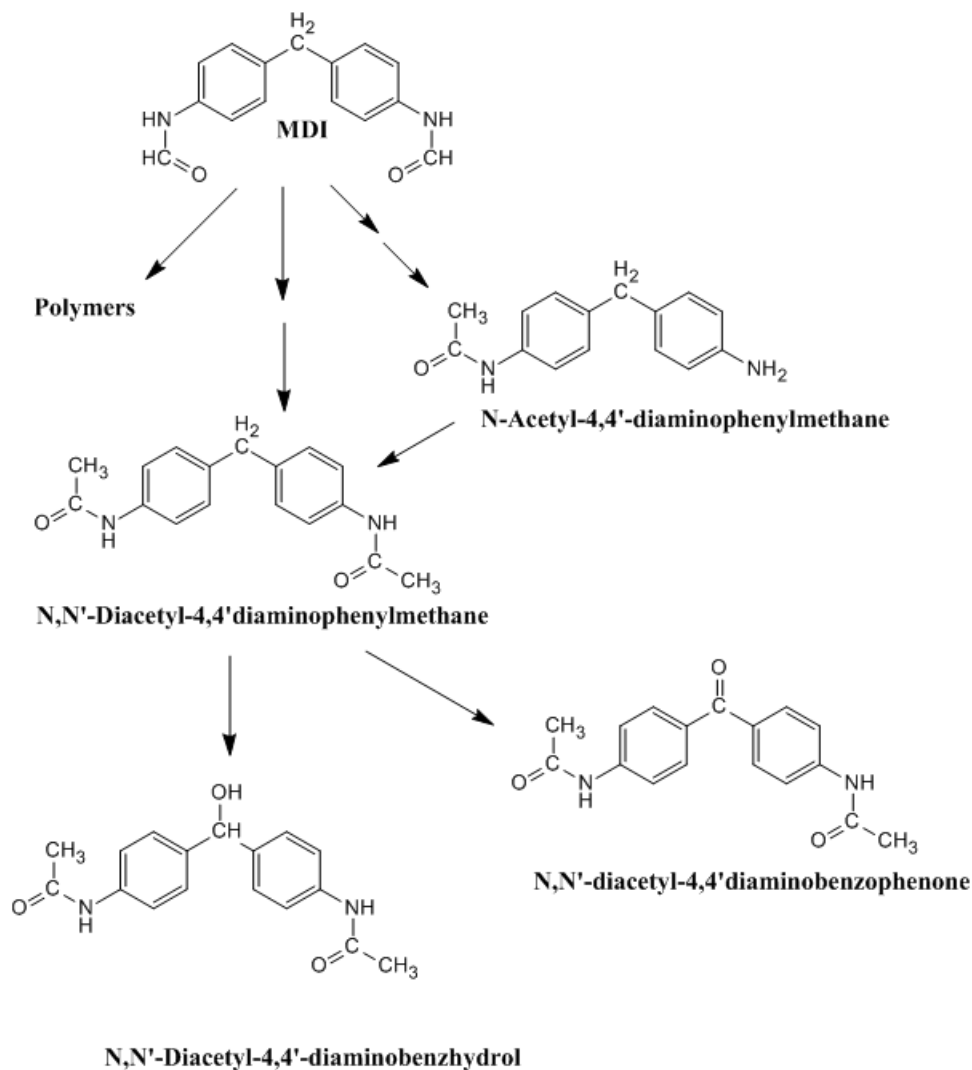
Methylene diphenyl diisocyanate (MDI) is used in the preparation of polyurethane resin and spandex fibers, and to bond rubber to rayon and nylon. Its use in polyurethane foams accounts for approximately 80% of the MDI consumed worldwide. With a vapor pressure of 5.0 x 10<sup>-6</sup> mm Hg at 25° C, MDI will exist in both the vapor and particulate phases in the ambient atmosphere. Vapor-phase MDI may be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals with an estimated half-life of 33 hours. Because MDI readily reacts with water, atmospheric degradation may also occur through contact with clouds, fog or rain. Particulate-phase MDI is removed from the atmosphere by both wet and dry deposition. Methylene diphenyl diisocyanate reacts rapidly with water to form amines and polyureas. As a result, MDI released to water or moist soil is expected not to leach or adsorb to solids, nor is it likely to accumulate in the food chain. While occupational exposure occurs through inhalation of vapors and aerosols, and through dermal contact with compounds containing MDI, the general population may be exposed during the use of MDI-containing urethane adhesives, coatings, and foam products. Exposure to particulate and/or vapor phase MDI may also result from thermal decomposition of MDI-containing polyurethane foam as may occur, for example, during manufacturing, structural fires, or welding of polyurethane insulated pipe. Reported release of MDI to the atmosphere in California in 2008 was at the rate of 3.6 tons per year (CARB 2008)

## 4. Metabolism

Given its high chemical reactivity, in the lungs MDI is expected to react initially with glutathione prior to being absorbed as the glutathione conjugate. Alternatively, a portion of the inhaled MDI may be cleared from the lungs and swallowed. If swallowed, conditions in the gastrointestinal tract favor spontaneous formation of polyureas, the smaller of which may be absorbed and excreted in the bile, while the larger urea polymers remain in the intestinal tract to be eliminated with the feces. The enzyme-catalyzed pathway of the proposed metabolic scheme (Figure 1) is expected to occur in the lungs, liver and/or kidneys following absorption and systemic distribution of MDI (Gledhill et al., 2005) with N-acetylation occurring prior to the hydroxylation step.

The metabolic pathway shown in Figure 1 features monomeric MDI. However, MDI is also commercially produced in a polymeric mixture (pMDI; Figure 2) usually consisting of 50% monomeric MDI with the remainder a mixture of oligomers containing three or more rings. It is not clear how and to what extent the metabolism of the polymeric and monomeric forms may be different.

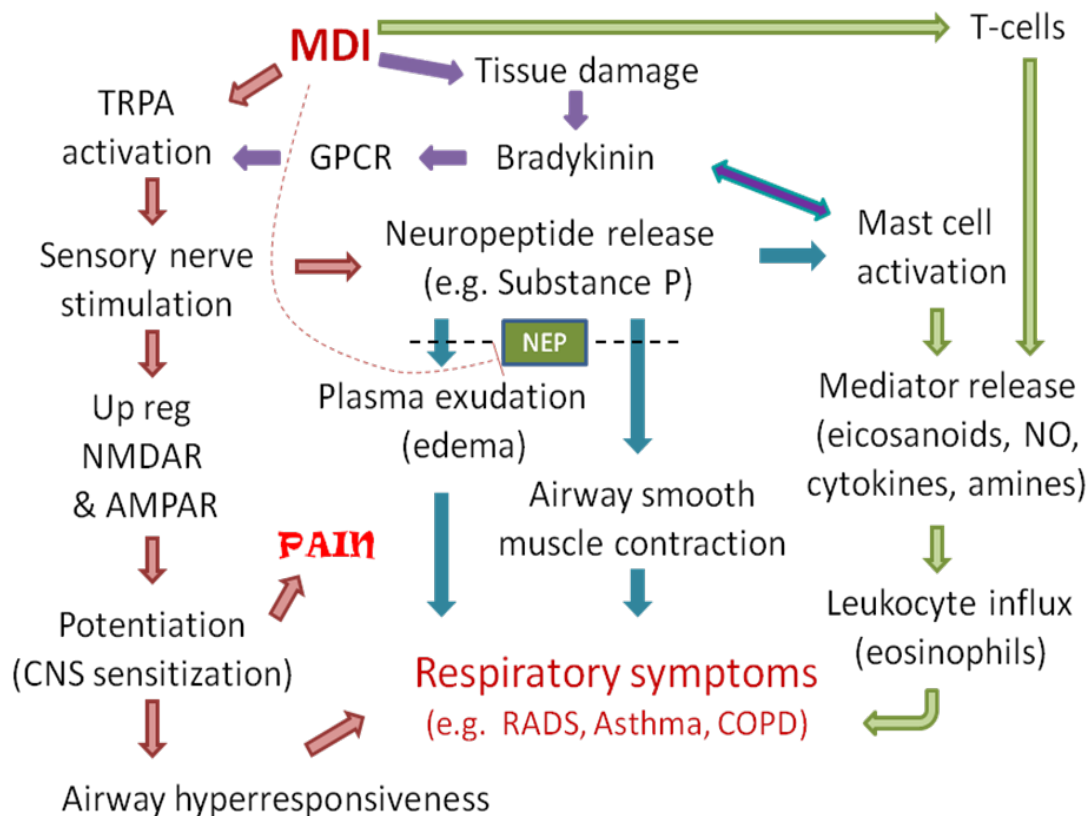
A study of genotypic variation in enzymes involved in the metabolism of MDI, specifically N-acetyltransferases (NATs) and glutathione transferases (GSTs), among occupationally exposed workers revealed a complex picture (Littorin et al., 2008). For example, two of four GST genotypes, GSTP1<sup>114</sup> and GSTP1<sup>105</sup>, were associated with higher levels of urinary metabolites of MDI than were the other two. At the same time, GST1<sup>105</sup> was associated with lower levels of serum MDI-specific IgG and fewer eye symptoms, but with an increased risk of symptoms in the airways, as well as with atopy. The allergic symptomatology appears to be affected by how rapidly MDI is conjugated to glutathione for excretion. By comparison, among workers with slow NAT2 acetylating capacity, lower plasma and urinary levels of MDI metabolites, lower MDI-specific IgG levels, and better lung function were observed, but with a higher risk of airway and eye symptoms. Thus the variations among workers in the manifestation of pulmonary and allergic symptoms following MDI exposure reflects the complex genotypic variation in metabolic enzymes, and the speed with which MDI is removed from the system.



**Figure 1 Metabolic Scheme for Monomeric MDI**

## 5. Acute Toxicity of Methylene-diphenyl diisocyanate

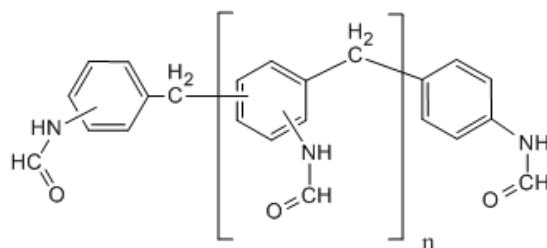
As is the case with other diisocyanates such as toluene diisocyanate (TDI), MDI has the capacity to cause sensitization of the neuroimmune system. Several isocyanates and other reactive electrophiles have been shown to activate cation channels of the transient receptor potential A (TRPA) group in sensory neurons (Macpherson et al., 2007; Taylor-Clark et al., 2009). This can lead to long term potentiation of synapses in the brainstem, and subsequent airway hyperresponsiveness (Figure 2). In addition, neuropeptides released during MDI stimulation of sensory neurons may cause mast cell degranulation, goblet cell hyperplasia and mucus secretion, contraction of airway smooth muscles, and pulmonary edema. However, MDI is less potent than TDI in causing these effects.



**Figure 2 Diisocyanate Pathways leading to respiratory symptoms**

Diisocyanates activate TRPA channels in nociceptive neurons in the airways leading to respiratory symptoms via long-term potentiation of neural pathways, release of inflammatory mediators, and stimulation of the immune system. NEP: neutral endopeptidase; GPCR: G-protein coupled receptor; NMDAR: N-methyl-D-aspartate receptor; AMPAR:  $\alpha$ -amino-3-hydroxy-5-methyl-4-isoxazolepropionic acid receptor.

As mentioned above, MDI is also commercially produced in a polymeric mixture with oligomers containing two or more rings (Figure 3). While the size of the oligomer may affect its deposition and distribution in the lungs, a review of studies of MDI and pMDI suggests that at least the pulmonary effects of the two forms are expected to be qualitatively similar (Feron et al., 2001).



**Figure 3 Structure of Polymeric MDI**

## 5.1 Toxicity to Adult Humans

Acute inhalation exposure to MDI generally results in irritation of the lungs and upper respiratory tract with symptoms including headache, sore throat, cough, and chest tightness. If the initial acute exposure is high enough, exposed individuals may become sensitized and, with subsequent low-level MDI exposures, exhibit pulmonary symptoms including bronchial hypersponsiveness and airflow obstruction (Leroyer et al., 1998).

In a case report, a foundry worker had frequent exposure to MDI but no reported respiratory or other symptoms. After three years he received an intense acute inhalation exposure as a result of an MDI spill in his work area. Within one hour he experienced headache, sore throat, cough and chest tightness. Other workers in the area experienced similar symptoms but only transiently. These initial symptoms were consistent with a diagnosis of reactive airways dysfunction syndrome (RADS). However, over the course of the subsequent month his chest symptoms and wheezing worsened, especially at work, with some remission during weekends. Spirometric testing revealed moderate airflow obstruction, an FEV<sub>1</sub> of 2.5 l (83% predicted), and an FVC of 4.5 l (121% predicted). Symptoms persisted despite treatment with budesonide, a glucocorticoid anti-inflammatory. After salbutamol inhalation (a  $\beta_2$ -adrenergic receptor agonist to treat bronchospasm), FEV<sub>1</sub> increased 12%. Bronchoprovocation tests with 15 ppb MDI were performed for 4, 30 and 60 min. An isolated late reaction was associated with the 60 min exposure, with a 22% fall in FEV<sub>1</sub> seven hours after exposure. The authors suggest his symptoms were consistent with occupational asthma caused by the acute high level exposure (Leroyer et al., 1998). However, it is not clear what role the low level exposures prior to the acute exposure may have played in the etiology of this case's symptomatology.

A similar pattern of symptoms among a small proportion of workers following acute high level exposure to spilled MDI has been reported elsewhere. Tanser et al. (1973) collected spirometric and health survey data from all 57 employees in a factory in which MDI was used extensively. Of 26 employees with routine contact with MDI, 12 reported symptoms including headache, chest tightness, sore throat, diarrhea, or wheezing. Among the 31 employees with only occasional MDI exposure, one reported mild, another, severe symptoms. Of the four most severe cases, one came into contact with molten MDI and vapor. While his initial contact caused wheezing, subsequent contacts caused severe wheezing lasting up to two hours. He was similarly affected outside the factory when in the vicinity of the extractor fan, and to a lesser extent while using polyurethane paints. The second case had worked for three weeks in the factory on a MDI/resin mixing machine when he complained of dyspnea and wheezing. His peak respiratory flow rate was 200 l/min but this improved to 450 l/min with choline theophyllinate. He returned to work in another part of the factory but found that he still became wheezy with occasional exposures to MDI. The third case did not use isocyanates directly but developed a fever, headache, cough and aching limbs when MDI was first used at the factory. On subsequent days when MDI was used in the factory, he would again experience symptoms of fever, headache, dyspnea, and loss of voice, lasting up to 48 hours. The fourth case complained of moderate but increasingly severe

wheezing with exposures to MDI. For most of the workers, MDI acted as a respiratory irritant. However, in the four cases described, a hypersensitivity to MDI is apparent. Of these, the first, second and fourth cases are consistent with a diagnosis of occupational asthma. Case three appears to reflect a delayed hypersensitivity reaction.

Unfortunately, since neither of these studies measured MDI levels, it is not known to what levels workers were routinely exposed, nor what the levels associated with MDI spills were. It appears, however, that acute exposure to the higher MDI levels associated with spills leads to an increase in the number and/or severity of respiratory symptoms, including asthma, following subsequent lower level exposures. This may suggest a sensitization response but it still is not clear whether a single high acute exposure is necessary or sufficient to elicit this sensitization.

## **5.2 Toxicity to Infants and Children**

Asthma-like symptoms were observed among 203 school children following acute exposure to MDI spilled during track paving with polyurethane (Jan et al., 2008). Of the exposed children, 70.9% reported headache, 67.5% had persistent cough, 63.5% had dyspnea, and 62.6% nausea. Chest discomfort was reported by 23.6% of the students but chest X-rays were normal. Bronchodilators were administered to 15.8% who experienced wheezing and difficulty breathing. Although no measurements of actual air MDI levels were reported, the authors observed an inverse linear relationship between the incidence of affected students in various classrooms and the distance from the site of MDI spillage ( $r = -0.48$ ,  $p < 0.05$ ) suggesting a dose-response. During follow-up surveillance three days after the incident, the prevalence of residual symptoms was cough 30.0%, headache 19.7%, dyspnea 15.3%, sore throat 10.3%, and nausea 3.9%. A positive history of asthma among 10.8% of the students was strongly correlated with the incidence of dyspnea (OR 4.09; 95% CI 1.17-14.32) and an abnormal pulmonary function test (OR 3.84; 95% CI 1.09-13.5). However, none of the other symptoms during the episode was correlated with either asthma history or abnormal lung function tests. In addition, 60.8% of the children without a history of asthma also complained of dyspnea, and 16.2% required bronchodilators for symptomatic relief. Acute exposure to high levels of MDI was thus associated with an asthma-like syndrome among previously unexposed individuals.

## **5.3 Toxicity to Experimental Animals**

During a single 4 hour exposure to high concentrations of pMDI aerosols (376 – 638 mg/m<sup>3</sup>, particle size < 5 µm), Wistar rats displayed labored respiration and mouth breathing (Reuzel et al., 1994). Deaths occurred at all exposure levels within two days following the end of exposure, with an LC<sub>50</sub> of 490 mg/m<sup>3</sup>. Among the animals that survived, transient weight loss was observed during the second and fourth days after exposure. At the higher aerosol levels, the lungs of rats euthanized immediately after exposure were grayish and wet, with some pulmonary hemorrhaging and hemorrhagic nasal discharge.

As with the acute exposures above, subacute exposure to pMDI aerosols (0, 2.2, 4.9, 13.6 mg/m<sup>3</sup>; 10 rats/sex/dose) for six hr/day, five days per week for two weeks led to labored respiration and mouth breathing in the high exposure group (13.6 mg/m<sup>3</sup>) starting on day four (Reuzel et al., 1994). Other clinical changes reported for this group included slow movements, dyspnea, piloerection, salivation, bleeding from the nares and swollen abdomens. While rats in the 2.2 mg/m<sup>3</sup> group were not visibly affected, those in the 4.9 mg/m<sup>3</sup> group were restless, slightly dyspneic, and showed piloerection. In all treatment groups, lung weights were elevated relative to body weights. The effects of pMDI were mainly on the respiratory tract with males more severely affected than females. However, in a subacute exposure to 14.1 mg/m<sup>3</sup>, rats that were four weeks old at the start of the exposures died earlier and in greater numbers than did rats that were six weeks old. This early life susceptibility was greater for females than for males. In all treatment groups with subchronic exposure there was significant (p < 0.05) accumulation of alveolar macrophages and, at the two higher concentrations, interstitial infiltration by macrophages. At all concentrations, there was also significant accumulation of yellowish pigmented macrophages in mediastinal lymph nodes. For these pulmonary effects, the authors reported a NOAEL of 1.4 mg/m<sup>3</sup>.

The adverse effects of acute exposures to pMDI manifest mainly in the lungs as pulmonary irritation characterized by increased immune cell infiltration, protein production, and organ weight. Kilgour et al. (2002) examined the appearance and resolution of these effects over a 30 day period in rats following an acute 6 hour exposure to 10, 30, 100 mg/m<sup>3</sup> pMDI. Immediately following a single, 6-hr acute exposure, lung lavage fluid showed massive increases in neutrophils (37% of total cells at 10 mg/m<sup>3</sup>, 78% at 100 mg/m<sup>3</sup>), but reduced numbers of alveolar macrophages. Protein content was elevated in lavage fluid, and enzyme activities increased for N-acetyl glucosaminidase (NAG), alkaline phosphatase, and lactate dehydrogenase (LDH). The accumulation of crystalline surfactant and cellular debris in alveolar lumina through day three at all pMDI concentrations suggests pMDI is cytotoxic to macrophages. By day three post-exposure, neutrophil numbers were still markedly elevated in the 100 mg/m<sup>3</sup> group, but had dropped substantially in the lower dose groups, while macrophage numbers increased. Lactate dehydrogenase continued to rise but the NAG and alkaline phosphatase activities had returned to control levels. By day ten, most of the measured parameters had returned to control levels, although epithelialization of the alveoli was observed in animals at 30 and 100 mg/m<sup>3</sup>. Thirty days following the last exposure, lung weights, lung lavage parameters, cell proliferation and ultrastructural appearance had returned to normal. These results suggest that even at relatively high acute exposure levels, recovery from pMDI-associated toxic effects in the lung is relatively rapid.

Many of the effects observed by Kilgour et al. (2002) may be related to MDI-induced changes in the pulmonary epithelium that forms the blood-air barrier in the lungs. Pauluhn (2000) examined bronchoalveolar lavage (BAL) fluid of rats for markers of damage to pulmonary epithelium following an acute 6-hr exposure to MDI at 0.7, 2.4, 8, or 20 mg/m<sup>3</sup>. These markers included angiotensin converting enzyme (ACE), protein levels, alkaline phosphatase, lactate dehydrogenase,  $\gamma$ -glutamyltranspeptidase, and sialic acid, and were assayed 3 hr, 1, 3, and 7 days following exposure. MDI at all dose levels

caused an immediate significant increase in alkaline phosphatase activity ( $p < 0.05$ ) that returned to control levels by day three. This was deemed to be consistent with an adaptive increase in pulmonary surfactant that is rich in alkaline phosphatase from type II pneumocytes. Plasma protein levels in BALF similarly were immediately and significantly elevated at all MDI concentrations suggesting damage to the epithelial barrier. The activity of ACE was also significantly elevated but only at  $2.4 \text{ mg/m}^3$  and above. However, there was not a dose-dependent effect on LDH levels, suggesting that cytotoxicity was not the cause of the elevated protein and ACE levels. Glutathione levels measured in BALF peaked on day one following exposure, and returned to control levels by day seven. However, GSH levels in lung tissue remained elevated through day seven. Based on these results, the authors propose that MDI interferes with the pulmonary surfactant system leading to surfactant dysfunction and increased alveolar surface tension. This surface tension in turn enhances transudation of fluid and solutes from the capillaries, contributing to the pulmonary edema characteristic of MDI exposure. While this effect appears to be transitory at these dose levels, it was observed to occur at concentrations as low as  $0.7 \text{ mg/m}^3$ .

## **6. Chronic Toxicity of Methylenediphenyl diisocyanate**

### **6.1 Toxicity to Adult Humans**

The effects of chronic exposure to MDI are largely reflected in decrements in pulmonary function and exacerbation of asthma. Impairment of lung function is mainly a function of allergic inflammation. When air sacs and lung parenchyma are affected, the result is extrinsic alveolitis, while involvement of the airways results in asthma. The symptoms of alveolitis include headache, nausea, muscle aches, fever and chills. This is in contrast to the variable airflow restriction and bronchial hypersensitivity associated with asthma. It is not clear from occupational studies whether these effects are more the result of chronic low-level exposure, acute exposure to high levels, or both. It is clear, however, that once individuals are sensitized to MDI, further exposure generally exacerbates respiratory symptoms (Vandenplas et al., 1993; Piirila et al., 2000).

In a prospective study of the respiratory effects of MDI exposure, Petsonk et al. (2000) evaluated the respiratory health of workers in a new wood products manufacturing plant in which MDI was used as a binder. Health data and exposure histories were collected by questionnaire prior to the use of MDI at the plant, and semiannually for the next two years. The critical effect was asthma, cases of which were defined as current or previous asthma, or current use of a bronchodilator, or current asthma attacks characterized by shortness of breath and wheezing. Cases were divided into those that met these criteria at the initial survey (IAS), those who met the definition during a follow-up survey (FAS; this presumably included most or all of those classified as IAS), and those meeting the definition only during follow-up. This third group was considered new onset asthma (NAS). Measurements of serial peak flow, spirometry, methacholine challenge, and specific IgE were used in some cases for validation of case designation, but were not available for all study participants. Of the 178 workers with initial and at least one follow-up survey, a complete occupational history was available for 144. Of these, 77

completed the initial health survey prior to the delivery of MDI. Thus the remaining 67 may have had MDI exposure at the plant prior to the initial health assessment.

The prevalence of FAS and NAS cases was clearly associated with reported exposure in that those who reported working with MDI were significantly more likely to have asthma ( $p < 0.01$ ) than were those with no or only occasional passing exposure. Those working in areas with high potential exposure to liquid MDI had a significantly elevated prevalence of asthma ( $p < 0.001$ ) compared to those where potential exposure was rated medium or low. Both FAS and NAS cases were significantly elevated among those who indicated they occasionally removed protective respirators compared with those who never did ( $p = 0.05$ ), and by 52% of those who reported at least once observing MDI stains on their skin ( $p < 0.001$ ). These observations in conjunction with the controls engineered into the plant's design to reduce inhalation exposure suggest that the appearance of new asthma symptoms among a third of those working in the blending and press operations, and 10-30% of the workers in adjacent areas, likely reflects both inhalation and dermal exposure to MDI. Relatively short-term exposures ( $< 2$  years) were sufficient to elicit symptoms of pulmonary dysfunction.

There are also case reports of neurological effects. In cases reported by Reidy and Butler (1994), five individuals were occupationally exposed to MDI over a two-year span, and examined while exposure was ongoing (1 case), or up to 9 months after cessation of exposure (4 cases). The intensity and frequency of exposure was not reported. Subjective complaints included respiratory distress, headaches, forgetfulness, mood alterations, irritability, and difficulty concentrating. Formal neuropsychological evaluations indicated that psychomotor, psychosensory, visuographic and language skills were largely intact. However, there was marked slowing in the rate of information processing, discrepancies in immediate recall of verbal versus nonverbal material, and deficiencies in learning ability. Complex, nonverbal abstract reasoning was impaired, and there was evidence of emotional distress in the form of depression, anxiety, and altered mentation. The symptomatology and test results were consistent with MDI-related damage to the CNS.

## **6.2 Toxicity to Infants and Children**

No studies of the effects on infants and children of chronic exposure to MDI were located.

## **6.3 Toxicity to Experimental Animals**

Chronic exposure to monomeric MDI causes a time and dose-dependent deterioration of lung function. Female Wistar rats were subjected to whole-body exposures to MDI aerosols (0.23, 0.70, 2.05 mg/m<sup>3</sup>; MMAD 1.03-1.06 μm) for 17 hours/day, 5 days/week for up to 24 months (Hoymann et al., 1998). Lung function was assessed after 6, 12, 17 and 20 months, with histological evaluations after 12 and 24 months. At all time points, the highest exposure (2.05 mg/m<sup>3</sup>) caused a significant decrease in maximum mid-

expiratory flow (MMEF), and forced expiratory flows (FEF) at 10, 25, and 50%, but not 75% of forced vital capacity. This indicates a significant increase in flow resistance in the small peripheral airways, but not the large airways. With the longer 12 and 17 month exposures, the decrements in these flow measures were seen at the lower doses as well. After 12 and 17 months at the high dose, the CO diffusion test showed a reduction in diffusion through the alveolar-capillary membrane. Lung weights associated with the high dose exposure were increased after 3, 12, and 20 months. In bronchoalveolar lavage fluid (BAL) obtained at these same time points, elevated hydroxyproline indicated increased collagen metabolism typically associated with fibrotic lesion formation in the lungs. Examination of BAL fluid also showed an inflammatory reaction, with increased numbers of lymphocytes, at all time points at the highest dose. These results are consistent with histopathologically determined dose-dependent interstitial and peribronchiolar fibrosis causing fibrotic thickening of walls of peripheral bronchioles and narrowing of small airways. The decline in lung function started before 6 months of exposure, increased through 12 months, and increased more slowly though 17 months. Measures of MMEF and FEF at 12 months suggest a LOAEL of  $0.2 \text{ mg/m}^3$ , the lowest dose used.

The effects of chronic exposure to pMDI (approximately 50:50 monomeric:polymeric MDI) of 560 Wistar rats of both sexes was reported by Reuzel et al. (1994). Animals were exposed to the pMDI mixture for 6 hr/day, 5 days/week for one or two years. The mean exposure concentrations were 0, 0.19, 0.98 and  $6.03 \text{ mg/m}^3$ , with mass median aerodynamic diameters of 0.68, 0.70, 0.74  $\mu\text{m}$ , respectively. After both one and two years of exposure, there was significant ( $p < 0.01$ ) accumulation of macrophages with yellow pigment in the lungs and the mediastinal lymph nodes at the highest dose ( $6.03 \text{ mg/m}^3$ ), and at  $0.98 \text{ mg/m}^3$  after two years of exposure. In the nasal cavity, minimal to moderate olfactory epithelial rearrangement was observed in all treatment groups, but was significant only at the highest dose. Basal cell hyperplasia and Bowman's gland hyperplasia were significant ( $p < 0.05$ ) at the two highest dose levels after two years. There was a significant incidence of benign pheochromocytomas and lung adenomas in males at the highest dose. These data indicate a LOAEL of  $0.98 \text{ mg/m}^3$ , and a NOAEL of  $0.19 \text{ mg/m}^3$  for these respiratory tract effects.

These results for pMDI were similar to those reported above for monomeric MDI by Hoymann et al. (1998) for which a NOAEL of  $0.23 \text{ mg/m}^3$  was suggested from an analysis by Feron et al. (2001).

## **7. Developmental and Reproductive Toxicity**

To examine the prenatal toxic effects of monomeric MDI aerosols, Buschmann et al. (1996) exposed pregnant Wistar rats to 0, 1, 3, and  $9 \text{ mg/m}^3$  for 6 hours per day on gestational days 6 to 15. At sacrifice on gestational day 20, lung weights were significantly increased in the high dose group ( $p < 0.01$ ), as were the number of litters with fetuses displaying asymmetric sternbra ( $p < 0.05$ ) (Table 1). These data were used in the derivation of the acute REL. Treatment reportedly had no effect on maternal weight gain, number of corpora lutea, implantation sites, pre- and post-implantation loss,

fetal and placental weight, gross and visceral anomalies, and degree of ossification. In the mid-dose range, slight deviations were observed in numbers of fetuses with dilated ureters, accessory lumbar ribs and incomplete ossification of sacral vertebral centers. The authors concluded that MDI up to concentrations of 1 mg/m<sup>3</sup> had no embryo- or fetotoxic effects. Maternal food consumption decreased at 3 mg/m<sup>3</sup> and maternal lung weights increased at 9 mg/m<sup>3</sup>. Buschmann et al indicate that a substance-induced effect on the sternbra cannot be ruled out at 9 mg/m<sup>3</sup> and suggest 3 mg/m<sup>3</sup> as a NOAEL for embryotoxicity.

**Table 1. Litters with Asymmetric Sternebra**

MDI (mg)	Litters (total)	Litter (assymmetric sternebra)	% Litters with anomalous fetuses
0	25	2	60
1	26	7	61.5
3	25	5	64
9	23	10*	69.6

\* p < 0.05

The prenatal effects of aerosols of the polymeric form of MDI were also examined in Wistar rats exposed on gestational days 6 through 15 to 0, 1, 4, and 12 mg/m<sup>3</sup> for 6 hours per day (Gamer et al., 2000). Maternal toxicity was clearly evident at the highest dose with significantly reduced body weight gain during pregnancy (p < 0.01) and, at sacrifice on day 20, significantly reduced organ and carcass weights. Fetal body weight per litter and placental weights per litter were also reduced at this dose (p ≤ 0.01 and p ≤ 0.05, respectively). Significant fetal toxicity manifested primarily at the highest dose and as skeletal malformations (p < 0.01). These included irregularly shaped sternbrae, bipartite sternbrae, and incomplete ossified vertebral bodies. Measured in terms of affected fetuses per litter, the incidences of total fetal variations were significantly increased in all exposed groups. The authors attribute this to an unexpectedly low incidence of variations in the control group, and suggest a NOAEL of 4 mg/m<sup>3</sup> for maternal and developmental toxicity of pMDI.

## 8. Derivation of Reference Exposure Levels

### 8.1 MDI Acute Reference Exposure Level

<i>Study</i>	Buschmann et al., 1996
<i>Study population</i>	Gravid Wistar rats
<i>Exposure method</i>	Whole body inhalation
	0, 1, 3, 9 mg/m <sup>3</sup>
<i>Continuity</i>	6 hr/day
<i>Duration</i>	Gestation days 6-15
<i>Critical effects</i>	Fetal skeletal anomalies
<i>LOAEL</i>	9 mg/m <sup>3</sup>
<i>NOAEL</i>	3 mg/m <sup>3</sup>
<i>BMCL<sub>05</sub></i>	0.49 mg/m <sup>3</sup>
<i>Time-adjusted exposure</i>	0.49 mg/m <sup>3</sup>
<i>Human equivalent concentration</i>	0.49 mg/m <sup>3</sup> RGDR =1 systemic effects
<i>LOAEL uncertainty factor</i>	Not applied
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>a-k</sub>)</i>	√10
<i>Toxicodynamic (UF<sub>a-d</sub>)</i>	√10
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>h-k</sub>)</i>	√10
<i>Toxicodynamic (UF<sub>h-d</sub>)</i>	√10
<i>Cumulative uncertainty factor</i>	100
<i>Reference Exposure Level</i>	5 µg/m <sup>3</sup> (0.49 ppb)

The acute REL is based on embryotoxicity reported in the developmental study by Buschmann et al. (1996). Specifically, the percentage of litters with fetuses showing skeletal anomalies was used in a benchmark concentration analysis (BMDS v. 1.4.1b). The data were well fit with the Weibull model ( $p = 0.999$ ). Setting the benchmark response rate to 5% gave a  $BMC_{05}$  of 1.70, and a  $BMCL_{05}$  of 0.49 mg/m<sup>3</sup>. In the absence of pharmacokinetic data on the developmental effects of MDI, no time adjustment is made and the 6-hr exposure is treated as one hour. Since the effects are systematic in nature, the default RGDR for the human equivalent concentration (HEC) adjustment is 1. To accommodate possible differences between rats and humans, the interspecies toxicokinetic and toxicodynamic UFs are both assigned √10. Similarly, the intraspecies UFs are also each assigned √10 to account for intra-individual variation. Since the study examined a highly sensitive life-stage, fetuses, higher intraspecies UFs are not required. The cumulative UF is thus 100 and the acute REL is 5 µg/m<sup>3</sup>.

## 8.2 MDI 8-hour Reference Exposure Level

<i>Study</i>	Reuzel et al., 1994a
<i>Study population</i>	240 Adult Wistar rats
<i>Exposure method</i>	Discontinuous whole-body inhalation exposure to 0-6.0 mg/m <sup>3</sup>
<i>Continuity</i>	6 hours per day, 5 days/week
<i>Duration</i>	104 weeks
<i>Critical effects</i>	Hyperplasia in olfactory epithelium
<i>LOAEL</i>	0.98 mg/m <sup>3</sup>
<i>NOAEL</i>	0.19 mg/m <sup>3</sup>
<i>BMCL<sub>05</sub></i>	0.25 mg/m <sup>3</sup>
<i>Time-adjusted exposure</i>	0.04464 mg/m <sup>3</sup> (0.25*6/24*5/7)
<i>Human equivalent concentration</i>	0.01486 mg/m <sup>3</sup> (0.0464*0.333 RGDR)
<i>LOAEL uncertainty factor</i>	Not applied
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>a-k</sub>)</i>	√10
<i>Toxicodynamic (UF<sub>a-d</sub>)</i>	√10
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>h-k</sub>)</i>	√10
<i>Toxicodynamic (UF<sub>h-d</sub>)</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Reference Exposure Level</i>	0.05 µg/m <sup>3</sup> (0.005 ppb)

The 8-hr REL is based on the observation of significant basal cell hyperplasia at the two highest doses in the olfactory epithelium of male rats, and at the highest dose in females, following two years of exposure ( $p < 0.05$ ) (Reuzel et al., 1994a). At these doses, there was also significant ( $p < 0.01$ ) accumulation of macrophages with yellow pigment in the lungs and in mediastinal lymph nodes, as well as degeneration of the olfactory epithelium. The benchmark concentration approach was applied to incidence data for both olfactory endpoints, basal cell hyperplasia and epithelial degeneration (Table 1; sexes combined), using US EPA BMDS v1.4.1b.

**Table 2 Incidence of Olfactory Hyperplasia and Degradation, Sexes Combined**

Exposure (mg/m <sup>3</sup> )	Hyperplasia	Degradation
0	18/120 (15%)	14/120 (12%)
0.2	21/120 (18%)	25/120 (21%)
1	34/120 (28%)	25/120 (21%)
6	81/119 (68%)	45/119 (38%)

Hyperplasia was selected as the critical endpoint as it represented an adverse response with a monotonic dose-response compared with epithelial degeneration. The quantal-linear and multistage models gave identical results with the best fit ( $p = 0.99$ ) and the

lowest Akiake Information Criterion (AIC = 508.896). They were used to estimate extra risk with the benchmark response rate set at the OEHHA default of 5%. The calculated BMD<sub>05</sub> and BMCL<sub>05</sub> were 0.31 and 0.25 mg/m<sup>3</sup>, respectively. Adjustment of the 6 hr/day, 5 day/week exposure to a daily 8-hour exposure would give a larger time-adjusted value (0.1339 mg/m<sup>3</sup> = 0.25\*6/8\*5/7 vs 0.04464 mg/m<sup>3</sup> = 0.25\*6/24\*5/7) than that indicated above. However, in consideration of the ability of MDI to cause neuroimmune system sensitization with repeated low level exposures, the time-adjusted value used is the same as for chronic exposures. This lower value was used to protect sensitized individuals. A dosimetric adjustment, using a regional gas dose ratio (RGDR) of 0.333, gave a human equivalent concentration of 0.04459 mg/m<sup>3</sup> as the point of departure. Toxicokinetic differences between rats and humans, and among humans are not expected to be large, so both the inter- and intraspecies UFs were assigned √10. Genotypic variation in MDI-metabolizing enzymes, described in Section 4 above, was addressed with an interspecies toxicodynamic UF of √10. The intraspecies toxicodynamic UF of 10 reflects both the genotypic variation in MDI metabolizing enzymes, MDI's sensitizing potential, and the greater susceptibility of children to the asthma-exacerbating effects of MDI described in Section 5.2. This gives a cumulative UF of 300, and an 8-hr REL of 0.05 µg/m<sup>3</sup>. This value is lower than the 0.13 µg/m<sup>3</sup> derived by Pauluhn (2008) as an 8-hr time-weighted average for non-sensitized individuals based on the elicitation of "asthma-like" responses in sensitized and naïve rats.

### 8.3 MDI Chronic Reference Exposure Level

<i>Study</i>	Reuzel et al., 1994a
<i>Study population</i>	240 Adult Wistar rats
<i>Exposure method</i>	Discontinuous whole-body inhalation exposure to 0-6.0 mg/m <sup>3</sup>
<i>Continuity</i>	6 hours per day, 5 days/week
<i>Duration</i>	104 weeks
<i>Critical effects</i>	Hyperplasia in olfactory epithelium
<i>LOAEL</i>	0.98 mg/m <sup>3</sup>
<i>NOAEL</i>	0.19 mg/m <sup>3</sup>
<i>BMCL<sub>05</sub></i>	0.25 mg/m <sup>3</sup>
<i>Time-adjusted exposure</i>	0.04464 mg/m <sup>3</sup> (0.25*6/24*5/7)
<i>Human equivalent concentration</i>	0.01486 mg/m <sup>3</sup> (0.0464*0.333 RGDR)
<i>LOAEL uncertainty factor</i>	Not applied
<i>Subchronic uncertainty factor</i>	1
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>a-k</sub>)</i>	√10
<i>Toxicodynamic (UF<sub>a-d</sub>)</i>	√10
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF<sub>h-k</sub>)</i>	√10
<i>Toxicodynamic (UF<sub>h-d</sub>)</i>	10
<i>Cumulative uncertainty factor</i>	300
<i>Reference Exposure Level</i>	0.05 µg/m <sup>3</sup> (0.005 ppb)

The chronic REL is also based on basal cell hyperplasia of the olfactory epithelium in the study by Reuzel et al. (1994a). As described above, the responses that attained significance included accumulation of macrophages with yellow pigment in the lungs and mediastinal lymph nodes, and basal cell hyperplasia and degeneration of the olfactory epithelium. The chronic REL uses the same benchmark analysis as the 8-hr REL with hyperplasia as the critical endpoint. The Quantal linear model was used to estimate the extra risk with the benchmark response rate set at the OEHHA default of 5%. The calculated BMD<sub>05</sub> and BMCL<sub>05</sub> were 0.31 and 0.25 mg/m<sup>3</sup>, respectively. Adjustment of the 6 hr/day, 5 day/week exposure to continuous exposure gave 0.04464 mg/m<sup>3</sup> (0.25\*6/24\*5/7). A dosimetric adjustment, using a regional gas dose ratio (RGDR) of 0.333, gave a human equivalent concentration of 0.01486 mg/m<sup>3</sup> as the point of departure. The uncertainty factors used and the rationale for their application are the same for the chronic and 8-hr RELs. Thus, both the inter- and intraspecies toxicokinetic UFs were assigned  $\sqrt{10}$ . The interspecies toxicodynamic UF of  $\sqrt{10}$  reflects genotypic variation in MDI-metabolizing enzymes. The intraspecies toxicodynamic UF of 10 includes both the variation in MDI metabolizing enzymes, the sensitizing potential of MDI, and the greater susceptibility of children to the asthma. This gave a cumulative UF of 300, and a chronic REL of 0.05  $\mu\text{g}/\text{m}^3$ . For comparison, the US EPA based its RfC of 0.6  $\mu\text{g}/\text{m}^3$  on a benchmark analysis of the same study. Whereas the RfC was based on data for males only, our analysis utilized the data for both sexes. Our analysis also uses a lower, more health-protective benchmark response rate (5% vs 10%), and an additional  $\sqrt{10}$  in the toxicodynamic UF specifically to protect against the onset of asthma symptoms in children.

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