OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Air Toxics Hot Spots Program

Hexamethylene Diisocyanate Reference Exposure Levels (Monomer and Polyisocyanates)

Technical Support Document for the Derivation of Noncancer Reference Exposure Levels

Appendix D1

Final

September 2019

Air, Community, and Environmental Research Branch

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency

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Appendix D1

Final Report

Prepared by the

Office of Environmental Health Hazard Assessment

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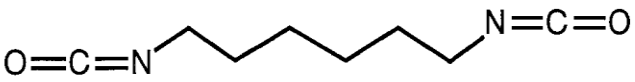
Hexamethylene Diisocyanate Reference Exposure Levels (Monomer and Polyisocyanates)

(1,6-Hexamethylene diisocyanate; 1,6-Diisocyanatohexane)

CAS No.: 822-06-0 (monomer)



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)). In response to this statutory requirement, OEHHA developed a Technical Support Document (TSD) that was adopted in 2008 and describes acute, 8-hour, and chronic Reference Exposure Levels (RELs). The TSD presents methodology for deriving Reference Exposure Levels. In particular, the methodology explicitly considers 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 RELs for hexamethylene-1,6-diisocyanate (HDI) and HDI-based polyisocyanate mixtures; this document will be added to Appendix D of the TSD.

Exposure to HDI monomer vapor and HDI-based polyisocyanate aerosols has been shown to cause adverse effects on the respiratory system of both animals and humans. These effects include: 1) acute sensory irritation and respiratory tract inflammation, 2) sensitization and induction of asthma with repeated exposure, and 3) decrements in lung function without evidence of sensitization following long-term exposure. Once asthma has been induced in HDI-sensitized individuals, triggering of attacks can occur following brief, low exposures (≤1 to 10 ppb) to the chemical. The RELs are intended to reasonably protect the public from these health effects resulting from exposure to HDI monomer and polyisocyanates, but may not protect all individuals previously sensitized to these compounds. Due to differences in potency and respiratory tract site of action, separate RELs have been derived for HDI monomer and polyisocyanate mixtures. Literature summarized and referenced in this document covers the relevant published literature for HDI through February, 2019.

1.1 Hexamethylene Diisocyanate Monomer Acute REL

Reference Exposure Level	0.3 µg/m^3 (0.04 ppb)
Critical effect(s)	Nasal epithelium lesions in rodents
Hazard index target(s)	Respiratory system

1.2 Hexamethylene Diisocyanate Monomer 8-Hour REL

Reference Exposure Level	0.06 µg/m^3 (0.009 ppb)
Critical effect(s)	HDI-induced asthma; accelerated decline in lung function in humans
Hazard index target(s)	Respiratory system

1.3 Hexamethylene Diisocyanate Monomer Chronic REL

Reference Exposure Level	0.03 µg/m^3 (0.004 ppb)
Critical effect(s)	HDI-induced asthma; accelerated decline in lung function in humans
Hazard index target(s)	Respiratory system

1.4 Hexamethylene Diisocyanate Polyisocyanate Acute REL

Reference Exposure Level	4.5 µg/m^3
Critical effect(s)	Increased total protein in pulmonary region of rodents
Hazard index target(s)	Respiratory system

1.5 Hexamethylene Diisocyanate Polyisocyanate 8-Hour REL

Reference Exposure Level	0.8 µg/m^3
Critical effect(s)	Pulmonary inflammation and fibrosis in rodents
Hazard index target(s)	Respiratory system

1.6 Hexamethylene Diisocyanate Polyisocyanate Chronic REL

Reference Exposure Level	0.4 µg/m^3
Critical effect(s)	Pulmonary inflammation and fibrosis in rodents
Hazard index target(s)	Respiratory system

List of Acronyms	
AEC Asymptomatic exposed controls	MMAD Mass median aerodynamic diameter
ACE Angiotensin converting enzyme	MMF Maximum mean expiratory flow
ACh Acetylcholine	NAT N-acetyl transferase
BHR Bronchial hyperresponsiveness	NCO Reactive isocyanate group
BALF Bronchoalveolar lavage fluid	NIOSH National Institute for Occupational Safety and Health
BMC Benchmark concentration	NOAEL No observed adverse effect level
BMC_{05} Benchmark concentration producing a 5% response rate	NOEL No observable effect level
$BMCL_{05}$ The 95% lower confidence limit of dose producing a 5% response rate	OA Occupational asthma
COPD Chronic obstructive pulmonary disease	OR Odds Ratio
CYP450 Cytochrome P-450	OSHA Occupational Safety and Health Administration
DA Diisocyanate-induced asthma	PD_{20} Provocation dose of methacholine (mg) to cause a 20% drop in FEV_1
$FEF_{25-75\%}$ Forced respiratory flow (25-75% of forced vital capacity)	PEFR Peak expiratory flow rate
FEV_1 Forced expiratory volume in 1 second	PMDI Polymeric methylene diphenyl diisocyanate
FVC Forced vital capacity	PMN Neutrophilic granulocytes
GSD Geometric standard deviation	POD Point of departure
GSH Glutathione	ppb Parts per billion
GST Glutathione-S-transferase	ppm Parts per million
HDA Hexamethylene diamine	RADS Reactive airways dysfunction syndrome
HDI Hexamethylene diisocyanate	RD_{50} Concentration resulting in a 50% depression of respiratory rate
HEC Human equivalent concentration	RDDR Regional deposited dose ratio
HLA Human leucocyte antigen	REL Reference exposure level
HSA Human serum albumin	RfC Reference concentration (US EPA)
IgE Immunoglobulin E antibody type	RGDR Regional gas deposition ratio
IgG Immunoglobulin G antibody type	SIC Specific inhalation challenge
LC_{01} Lethal concentration for 1% of animal test population	SNP Single nucleotide polymorphism
LC_{50} Median lethal concentration	TDI Toluene diisocyanate
LDH Lactate dehydrogenase	TLV Threshold limit value
LOAEL Lowest observed adverse effect level	TRIG Total reactive isocyanate group
LOD Limit of detection	TSD Technical support document
MAMA 9-N-methyl-amino-anthracene	TWA Time-weighted average
MDI Methylene diphenyl diisocyanate	UF Uncertainty factor
	VC Vital capacity
	VOC Volatile organic compound

2. Physical & Chemical Properties

Sources: (SIDS, 2001; HSDB, 2016)

HDI Monomer:

Description	Clear colorless to slightly yellow liquid
Molecular formula	$C_8H_{12}N_2O_2$
Molecular weight	168.20 g/mol
Density	1.04 g/cm^3 (25°C)
Boiling point	213°C
Melting point	-67°C
Vapor pressure	0.05 mm Hg (25°C); 0.7 Pa (20°C)
Saturated vapor pressure	30 ppm (25°C)
Odor threshold	Pungent; threshold 0.001 ppm
Solubility	Poorly soluble in water; slowly reacts with water to form CO_2; reacts with alcohols; soluble in organic solvents
Conversion factor	1 ppm = 6.879 mg/m^3 (25°C) 1 mg/m^3 = 0.145 ppm (25°C)

HDI monomer is primarily processed into higher molecular weight compounds with similar reactivity, but with lower volatility and potential for inhalation exposure. Products containing these compounds still have a residual amount (<1-2%) of the HDI monomer (Fent *et al.*, 2008; Reeb-Whitaker *et al.*, 2012). Figure 1 shows the most common HDI-based compounds, often referred to as “prepolymers”, which are found in HDI polyurethane paint-related formulations. Only limited physical and chemical property information could be located for these compounds. Prepolymers are reaction products of polyols with a stoichiometric excess of diisocyanates or polyisocyanates. The main HDI-based prepolymers used in polyurethane paints are the isocyanurate, biuret, and uretidone (also known as uretdione) forms of HDI. “Polyisocyanates” is the term often used to refer to a mixture of the diisocyanate monomer and various higher molecular weight diisocyanate reaction products, such as HDI prepolymers, which are found in polyurethane paint. Polyisocyanates have two or more reactive isocyanate (NCO) groups per molecule. Other HDI-based prepolymers include the asymmetric trimer of HDI marketed under the trademark Desmodur XP 2410, and the isocyanurate copolymer of toluene diisocyanate and HDI marketed under the trademark Desmodur HL (Bayer MaterialScience, 2005). The term “isocyanate oligomer” is also used and refers to relatively low molecular weight polyisocyanates, containing up to 10-15 monomeric units. The term isocyanate is used here to refer to those isocyanate group-containing compounds that can induce asthma in susceptible individuals with sufficient exposure. This includes compounds containing two (i.e., diisocyanates) or more isocyanate groups. The bivalent nature and intra-molecular cross-linking ability of these compounds with proteins and peptides are thought to be involved in asthma

pathogenesis (Wisnewski et al., 2015; Redlich and Karol, 2002). Monoisocyanates, such as methyl isocyanate, are also highly reactive, but are not included in this definition. Compounds containing one isocyanate group are not cross-linking agents or known sensitizers (Redlich et al., 2007).

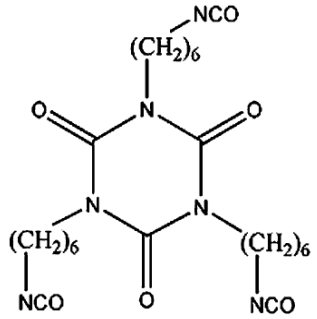
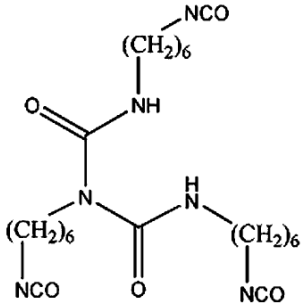
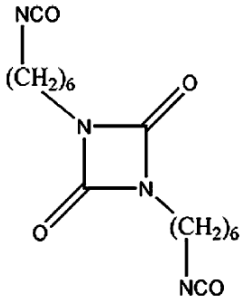
HDI Prepolymer	Properties	Chemical Structure
Isocyanurate	CAS: 3779-63-3 A ring condensate Molecular Weight: 504.7 g/mol Vapor Pressure: 5.2E-9 mmHg (20°C)	
Biuret	CAS: 4035-89-6 A condensation product of HDI and water Molecular Weight: 478.7 g/mol Vapor Pressure: 4.7E-7 mmHg (20°C)	
Uretidone	CAS: unknown Molecular Weight: 336.4 g/mol Vapor Pressure: unknown	

Figure 1. HDI prepolymers found in HDI-based polyurethane formulations (Fent, 2008).

3. Major Uses and Sources

HDI-based polyisocyanates are primarily used as hardeners for automobile and airplane polyurethane spray paints, including primers, sealers, and clear coats (Fent *et al.*, 2008). Coatings using HDI-based polyisocyanates have high resistance to UV light and good colorfastness and gloss retention, resulting in these isocyanate compounds being preferred under outdoor weathering conditions (Bayer MaterialScience, 2005; Porto,

2015). Other sources of these compounds include coatings primarily for outdoor furniture, parquet and industrial wood, and architectural finishing (SIDS, 2001; Dow Chemical Co., 2010). HDI polyisocyanates may also be used in some adhesives, and in elastomeric waterproof layer applications for parking decks, bridges and marine decks (Dow Chemical Co., 2010; Covestro LLC, 2015b) .

Other common isocyanate compounds used to manufacture polyurethane products include toluene diisocyanate (TDI) and methylene diphenyl diisocyanate (MDI). TDI is primarily used to make flexible polyurethane foams, but is also found in adhesives, sealants, and coatings (Covestro, 2019). Similar to HDI, TDI is used in coatings for furniture and other wood products. Due to poor resistance to UV light exposure, wood coatings made with TDI are largely relegated to indoor uses (Bayer MaterialScience, 2005; Porto, 2015). MDI is used for the production of rigid polyurethane foams that are good thermal insulators in homes and buildings, and refrigerators and freezers (Covestro, 2019). MDI also is used for some types of nylon fibers and in high-strength adhesives.

Industrial hygiene concerns due to the high vapor pressure of monomeric HDI limit its use in most typical coatings applications (Bayer MaterialScience, 2005). HDI polyisocyanate formulations, consisting mainly of a mixture of several different low vapor pressure prepolymers, reduce exposure concerns associated with manufacturing and handling of polyurethane coatings. Environmental exposures may occur from emissions of facilities that manufacture HDI, or from emissions in the immediate vicinity of small end-use applications such as automobile paint-spraying facilities (Greenberg and Foureman, 1995).

Monomeric HDI in the atmosphere exists primarily in the vapor phase. However, the tendency of HDI to partition between vapor and aerosol/condensed phases in paint products is a function of concentration, method of generation, and inherent volatility (Rando and Poovey, 1999). In HDI-based polyisocyanate paints containing mainly biuret and <1% HDI monomer, aerosolization under paint spraying conditions results in the monomer partitioning approximately 80% in the vapor phase and 20% as aerosol, whereas biuret partitions only into the aerosol phase. In another study, sampling of overspray from polyurethane paints being applied to automobiles found that the fraction of HDI monomer in aerosol form was 57 ± 9.4% (Fent *et al.*, 2008). The HDI prepolymers in the overspray existed primarily in the aerosol phase.

The US Environmental Protection Agency (US EPA) reported in its Inventory Update Reporting (IUR) that the aggregated national production volume of HDI was 50 million to 100 million lbs in 2006 (U. S. EPA, 2010). The California Toxics Inventory (CTI) provides total organic gas and particulate matter emission estimates by stationary (point

and aggregated point), area wide, on-road mobile (gasoline and diesel), off-road mobile (gasoline, diesel, and other), and natural sources. The reported CTI stationary source emissions of HDI in 2006, 2008, and 2010 were 0.08, 0.08, and 0.4 tons/year, respectively (CARB, 2013) Stationary sources include point sources provided by facility operators and/or districts pursuant to the Air Toxics "Hot Spots" Program (AB 2588), and aggregated point sources estimated by the Air Resources Board (ARB) and/or local air districts. These emissions are only for HDI monomer. No information was provided for emissions of HDI polyisocyanates.

US occupational health values are available for HDI monomer and HDI polyisocyanates. For monomeric HDI, the American Conference of Governmental Industrial Hygienists (ACGIH) lists a Threshold Limit Value-Time-Weighted Average (TLV-TWA) of 0.005 ppm (Cassidy *et al.*, 2010). The National Institute for Occupational Safety and Health (NIOSH) has a 0.005 ppm (0.035 mg/m^3) TWA 8 hr health value, and a 0.02 ppm (0.140 mg/m^3) short-term (10 min TWA) ceiling limit (NIOSH, 2015). Ceiling limits are applied to irritants and other materials that have immediate adverse health effects and are not to be exceeded for any period of time. The California Occupational Safety and Health Administration (CalOSHA) has a similar daily TWA Permissible Exposure Level (PEL) of 0.005 ppm, while US OSHA does not have an occupational exposure value for monomeric HDI (California OSHA, 2016). Currently, only the Oregon OSHA has an occupational exposure level for HDI polyisocyanates. An 8-hr TWA of 0.5 mg/m^3 and an acceptable ceiling concentration of 1.0 mg/m^3 has been developed for HDI diisocyanate-based adduct, including HDI-biuret, isocyanurate, and other polymeric forms of HDI (Oregon OSHA, 2016).

Internationally, occupational exposure limits for all isocyanates (as total reactive isocyanate group, in mg NCO group per m^3) have been established, which would include HDI in its various forms. In the United Kingdom and Australia, 8-hour and short term limit values of 0.02 and 0.07 mg/m^3, respectively, are listed for all isocyanates. In Switzerland, both the 8-hour and short-term values for all isocyanates is 0.02 mg/m^3 (GESTIS, 2019).

Airborne emissions of HDI monomer and prepolymers in overspray during spray paint operations are a primary source for occupational exposure (Butcher *et al.*, 1993). Fent *et al*. (2009) used one- and two-stage samplers to determine breathing zone concentrations of both gas and aerosol forms of HDI isocyanates during paint spraying tasks. A liquid chromatography/mass spectrometry method was used to quantify HDI and its main prepolymers (uretidone, biuret, and isocyanurate) in the collected samples (Table 1). Isocyanurate was the most abundant component measured in paint formulations and in air of ventilated booths during application of clear coat, although

individual HDI prepolymer concentrations in paints and air varied considerably between paint formulations.

Table 1. Assessment of exposure to monomeric HDI and HDI-based prepolymers in automotive spray painters (Fent *et al.*, 2008)

Analyte	Air Sampling (n=34) Breathing Zone Concentration µg/m^3		Paint Sampling (n=34) Paint Concentration mg/L	
	Mean	**Range**	**Mean**	**Range**
HDI	20.2	nd-179	202	nd-530
Uretidone	17.2	nd-124	2150	nd-17,000
Biuret	609	nd-7730	1760	nd-23,800
Isocyanurate	3540	7.06-17,800	52,800	3980-154,000

nd – Non-detectable

Similar quantification results in overspray from spray paint operations were found by Reeb-Whitaker *et al.* (2012) in Washington State auto body shops. The maximum percentage of specific prepolymers in the air samples of paint booths ranged from 58% biuret to 96% isocyanurate. However, HDI and the three prepolymers shown in Figure 1 are not the only isocyanates present in paint formulations. Small amounts of di-, tri-, and tetra-HDI oligomers are also found in paint samples and workplace air during spray painting, as well as tri-, and tetra-HDI-isocyanurate oligomers (Marand *et al.*, 2004). Although not the focus of this REL summary, isophorone diisocyanate (IPDI) and its oligomers are also constituents in some paint formulations and would be expected to have many of the same health concerns as HDI polyisocyanates (Woskie *et al.*, 2004).

Particle sizes of HDI-polyisocyanate-based paints aerosolized during spraying operations have been measured. In an impinger-filter sampling method used by Marand *et al*. (2004), the impinger collected large spray particles (>1.5 µm) and gas phase chemicals, while the filter collected particles in the range of 0.01-1.5 µm. In paint spray applications using HDI-based polyisocyanates, most of the spray particles were found in the impinger, indicating the particles are >1.5 µm. During a simulated spray booth operation, particle sizes were measured with a fixed sampling probe connected to a laser-particle counter (Maitre *et al.*, 1996b). Particle size was dependent on the type of paint used. For a HDI-isocyanurate-based paint, 17% by weight was <1.5 µm aerodynamic diameter, and more than 90% by weight was represented by particles <3 µm aerodynamic diameter. For a HDI-biuret-based paint, only about 2% by weight was <1.5 µm aerodynamic diameter, and <27% by weight was represented by particles <3 µm aerodynamic diameter. For both paints, few of the particles were >10 µm aerodynamic diameter. For biological relevance, Brown *et al*. (2013) estimated 50% cut-points for particle penetration beyond the larynx at ~3 µm in adults and ~5 µm in

children. The predicted 50% cut-points for particle penetration beyond the ciliated airways were slightly less than 3 µm in adults and slightly greater than 4 µm in children.

HDI released to the environment is expected to break down and not accumulate in the environment (Covestro LLC, 2015b). HDI vapor released to air will degrade in the air with a half-life of about 5.6 hrs. Aliphatic isocyanates such as HDI and HDI prepolymers are poorly soluble or insoluble in water (Covestro LLC, 2015a). However, the isocyanate group will react slowly at room temperature with water to form insoluble white solids (i.e., ureas) and release carbon dioxide gas. Heating or mixing will result in a more rapid reaction.

Determining the total number of reactive isocyanate groups (TRIG) for HDI polyisocyanate exposure, rather than specific isocyanate species, is another method to assess exposure and toxicity (Bello *et al.*, 2004; Woskie *et al.*, 2004; Reeb-Whitaker *et al.*, 2012). The isocyanate NCO functional group metric, often expressed in µg NCO/m^3, offers advantages such as consistency of calculation across all types of isocyanates and the ability to quantify exposures to material comprised of a mixture of isocyanate products (e.g., HDI polyisocyanates).

However, the TRIG approach may not work in all cases for assessing toxicity. For example, a study by Pauluhn (2004) suggests that the content of free NCO was a better predictor of the pulmonary irritant potency for a series of HDI-based polyisocyanate aerosols, but not between different types of isocyanates (e.g., aromatic vs. aliphatic polyisocyanates). Roughly ten-fold or greater differences in pulmonary irritation thresholds in rats, measured largely as increased total protein and LDH in bronchoalveolar lavage fluid (BALF), were found for various HDI, TDI and MDI polyisocyanate mixtures even though the percent content of free NCO varied less than 3-fold (free NCO range: 11 to 31%) among these polyisocyanates.

HDI and its prepolymers have relatively high flash points and are not considered flammable (Bayer Corp., 2002; Bayer MaterialScience, 2013). However, if heated sufficiently, thermal decomposition of materials made from HDI polyisocyanates are expected to release HDI monomer vapors and other toxic fumes and gases, such as carbon monoxide, carbon dioxide, oxides of nitrogen, hydrogen cyanide, and isocyanic acid. Thermal degradation of polyurethane material in house and car fires represents a hazard to firefighters and other individuals that may be exposed (Blomqvist *et al.*, 2003; Boutin *et al.*, 2004; Lonnermark and Blomqvist, 2006; Fent and Evans, 2011). Bench-scale tests are predominantly used to identify fire gases generated from polyurethane materials, mainly because large-scale fire tests (e.g., car and house fires) are relatively expensive, difficult to reproduce, complex to set up, and lack standardized scenarios or procedures (Bengtstrom *et al.*, 2016).

The heating of various polyurethane coating samples to temperatures in the range of 100-500°C showed that at temperatures <350°C release of monomers such as HDI, TDI, and IDPI occurred, whereas at temperatures >350°C monoisocyanates dominated (Karlsson *et al.*, 2000). Only about 0.1-1.0% of the total weight emitted during thermal degradation was identified as isocyanates. Boutin *et al*. (2005) determined levels of the eight most abundant isocyanates generated following thermal degradation of polymerized clear coat car paint primarily consisting of HDI isocyanurate. Heating of car paint samples resulted in temperatures reaching 475°C. Emission yields of isocyanic acid and HDI monomer were 6.24 and 3.58 mg/g of degraded polymer, respectively. Emission yields for the other six isocyanates, methyl-, ethyl-, propyl-, butyl-, pentyl-, and hexyl-isocyanate, each were in the range of 0.36 to 1 mg/g of polymer degraded.

Fent and Evans (2011) measured the breathing zone concentrations of isocyanates and other fire gases during the suppression of three vehicle fires. Hazard quotients were then calculated by dividing the predicted and measured concentrations by the most conservative worker short-term exposure limits (STELs) or ceiling limits. Isocyanate concentration, including HDI, TDI, MDI and other isocyanates, was reported as TRIG (i.e., the sum of masses of all isocyanate functional groups in a sample). TRIG was only detected in cabin fires, and not engine fires, which was expected since polyurethane foams are found in the interior of the vehicles, and not the engine. The predominant contributors to the hazard index (i.e., the sum of hazard quotients) for eye and respiratory tract irritation/injury was TRIG, formaldehyde and acrolein, which combined, were well above the level of concern.

Field measurements taken during grinding, cutting, and welding operations in car repair shops observed the release of HDI monomer concentrations up to 8 µg/m^3, most of which was in vapor form (Karlsson *et al.*, 2000). Emissions of TDI and MDI also occurred due to their presence in glues, soft and rigid foams, under-body coatings and elastomers. The particle size distribution of emitted isocyanates during grinding, cutting or welding processes were similar, with particles mostly in the 0.02-0.1 µm range. When expressed as total reactive isocyanate group (NCO) mass in unit volume of air, Henriks-Eckerman *et al*. (2002) measured personal air levels as high as 2.8 µg NCO/m^3 of HDI during grinding and welding operations in auto repair shops. MDI was also detected in the breathing zone of workers during these processes when polyurethane foam insulation made with MDI was burned. Isocyanic acid and methyl isocyanate were also detected, but only during welding operations.

Occupational exposure of skin to HDI polyisocyanates is common in spray painting operations and may contribute to sensitization and asthma (Liu *et al.*, 2000; Bello *et al.*, 2008). Animal studies show that repeated skin exposures to HDI polyisocyanates are

effective at inducing sensitization, with subsequent inhalation challenge eliciting an asthmatic response (Pauluhn, 2015). Dermal exposure to HDI polyisocyanates is an important consideration for occupational exposure, but is unlikely under a Hot Spots exposure scenario. The short half-life of HDI polyisocyanates in the environment and consequent lack of accumulation potential on soil and other surfaces indicate that the dermal route would not be a significant source of exposure in Hot Spots assessments.

4. Metabolism

Isocyanates are highly reactive with primary and secondary amines to form urea compounds, and with alcohols and phenols to form urethane compounds. HDI is one of the most commonly used diisocyanates within the family of aliphatic isocyanates. Its reactivity with active hydrogen compounds is orders of magnitude less than aromatic isocyanates (e.g., TDI and MDI) as a result of the electrophilic effect of the aromatic ring on the N=C=O bond (Bello *et al.*, 2004). This difference suggests a lower toxicological potency among aliphatic isocyanates compared to aromatic isocyanates. However, other properties such as lipophilicity, three-dimensional structure, and lung deposition site are likely contributors to toxicity differences among isocyanates.

HDI Monomer Metabolism Studies

Currently, there is little information on HDI prepolymer metabolism, so HDI monomer metabolism is used as a surrogate for metabolism of HDI prepolymers. When inhaled, HDI-protein adducts form as a result of conjugation to various macromolecules at the site of contact within the respiratory system (Redlich *et al.*, 1997). It has been postulated that HDI-glutathione conjugates formed in the lung lining fluid may be shuttled through the body in a reactive form, and subsequently conjugate to proteins distant from the lungs (Wisnewski and Redlich, 2001). In the bloodstream, albumin and hemoglobin adducts have been identified following exposure to HDI (Wisnewski and Redlich, 2001; Flack *et al.*, 2010b; Flack *et al.*, 2011). Albumin has a half-life of 19 days, while hemoglobin has a lifespan of ≤120 days. Thus, HDI-hemoglobin adducts may be broken down and eliminated over a longer period than HDI-albumin adducts.

Studies have examined the metabolism of HDI and other diisocyanates in humans. A metabolism scheme for HDI has been proposed by Flack *et al*. (2010a) (see Figure 2): Albumin or hemoglobin adducts in blood (Fig. 2, [1A]) may result from direct interaction of HDI (Fig. 2, [1]) with blood proteins. HDI can also react spontaneously or through catalysis by glutathione-S-transferase (GST) to form mono- and bis-dithiocarbamate adducts (Fig. 2, [2]). HDI-protein conjugates are also created via the base-catalyzed elimination reaction of the bis-dithiocarbamate adducts to form an intermediate S-glutathionyl adduct (Fig. 2, [3]), which can undergo carbamoylation reactions with

proteins or undergo solvolysis to regenerate HDI (Fig. 2, [1]), resulting in potential carbamoylations with other nucleophilic sites on albumin or hemoglobin far from the initial site of contact (Fig. 2, [2A]). This reaction pathway competes with the base-catalyzed biomolecular substitution reaction, leading to the formation of the highly labile carbamic acid group (Fig. 2, [4]), which decomposes to hexamethylene diamine (HDA) (Fig. 2, [5]).

An alternative pathway for diisocyanate-adduct formation is based on the N-hydroxylation of the amines (Fig. 2, [5,6]), catalyzed by cytochrome P-450 isoforms, resulting in the formation of N-hydroxyamine (Fig. 2, [8]) and the nitroso compound (Fig. 2, [9]), which can react with thiols on albumin or hemoglobin (Fig. 2, [3A]). This pathway competes with N-acetylation of the amine, catalyzed by N-acetyl transferase (NAT) enzymes, to form monoacetylated (Fig. 2, [6]) and diacetylated amines (Fig. 2, [7]), which are excreted in urine.

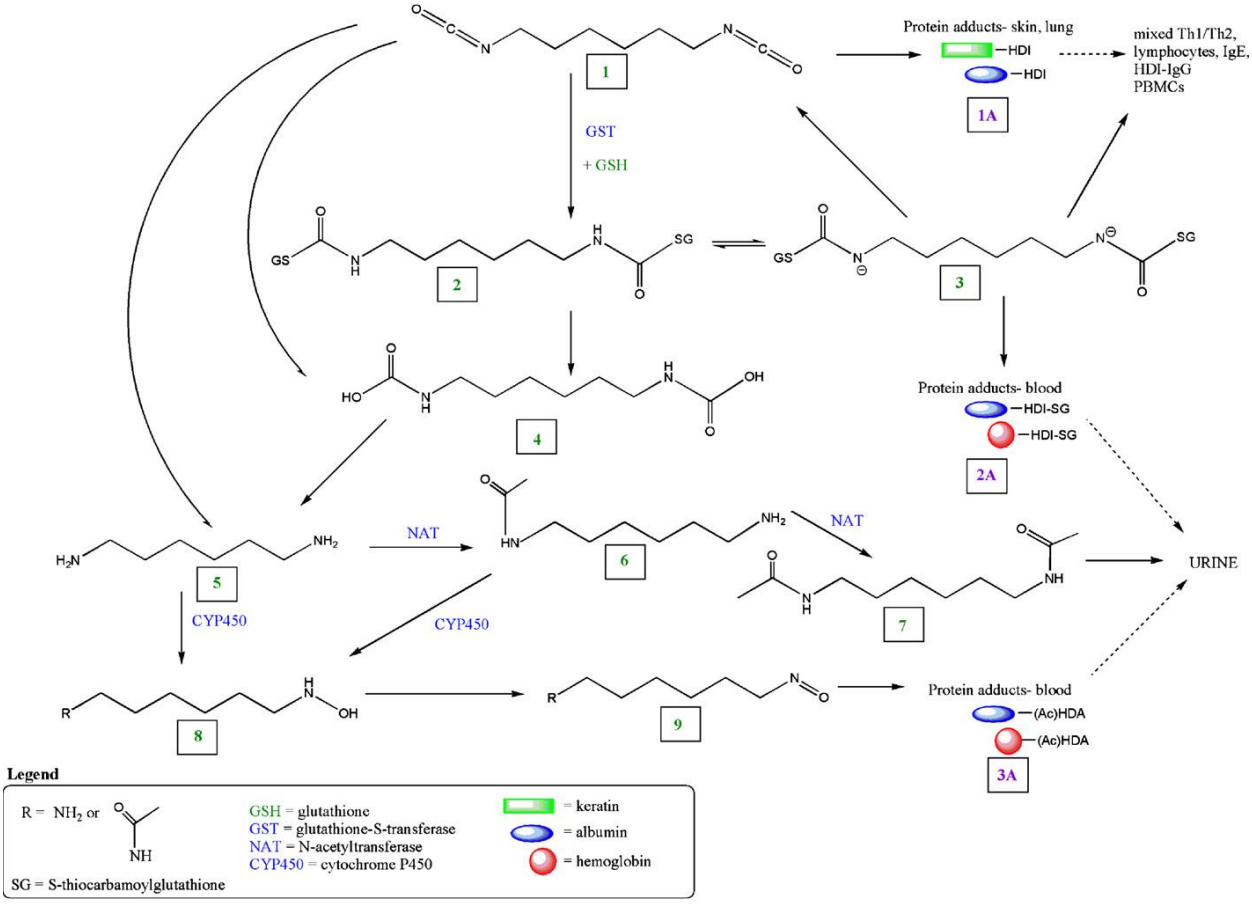


Figure 2. Proposed enzymatic (GST, NAT, CYP450) and non-enzymatic HDI metabolic pathways leading to formation of HDI–protein adducts, potential immune response, and elimination (Flack *et al.*, 2010a). [1] – HDI, [1a] – keratin and albumin adducts of HDI, [2] - bis-(or mono-) dithiocarbamate GSH adduct, [2a] – albumin and hemoglobin adducts of HDI S-

thiocarbamoylglutathione, [3] - intermediate S-glutathionyl adduct, [3a] - albumin and hemoglobin adducts of monoacetylated form of HDA [4] - very labile carbamic acid form, [5] - hexamethylene diamine (HDA), [6] – monoacetylated form of HDA, [7] – diacetylated form of HDA, [8], N-hydroxyamine form of HDA, [9] – nitroso form of HDA.

As illustrated in Figure 2, HDI and other isocyanates can bind to albumin and several other human proteins. The bivalent nature and cross-linking ability of HDI may alter the structure of albumin to transform the isocyanate-albumin conjugate into an antigenic carrier protein that presents or participates in the presentation of HDI to the immune system (Wisnewski and Redlich, 2001; Flack *et al.*, 2010a). This process results in a mixed Th1/Th2 type response and lymphocyte production. Peripheral blood mononuclear cells (PBMC) have been shown to be innately activated by HDI-albumin conjugates both *in vitro* and *in vivo* (Wisnewski *et al.*, 2008; Wisnewski and Jones, 2010). The monocyte is the main PBMC type that produces the histamine releasing factor MCP-1. This chemokine has equal or greater histamine releasing activity than that triggered by immunoglobulin E antibody (IgE) receptor cross-linking. Therefore, innate immune cells may have a role in isocyanate-induced diseases.

Wisnewski et al. (2013) has shown *in vitro* that GSH may act as a “shuttle” for MDI (and other diisocyanates including HDI), in which diisocyanate is transported from the airways to the blood, where albumin is the dominate reactant protein. Once MDI-GSH is absorbed, MDI-albumin conjugates can be generated via GSH-mediated transcarbamoylation that then cause distinct changes in conformation and charge. These MDI-albumin conjugates were specifically recognized by serum IgG of MDI workers with iisocyanate-induced asthma, suggesting one possible pathway for MDI in promoting immune responses.

Several HDI monomer inhalation studies have been conducted in humans to quantify the resulting HDI metabolites in urine and plasma. However, only one study by Liu *et al*. (2004) investigated urinary metabolites of inhaled HDI prepolymers in human subjects. For routine analysis of HDI metabolites, the determination of HDA after acid treatment of urine or plasma samples from exposed subjects is considered the most sensitive method of detection and is used as the biomarker of choice for HDI exposure. The total HDA concentration measured in biological samples represents the sum of covalently bound HDI monomer and HDA/monoacetylated HDA oxidation products, as well as non-covalently bound HDA and its metabolites (e.g., mono- and di-acetylated HDA).

Three volunteers were chamber-exposed to HDI on three different occasions at concentrations of 11.9, 20.5 or 22.1 µg/m^3 (1.7, 3.0 and 3.2 ppb, respectively) for 2 hrs each (Tinnerberg *et al.*, 1995). Exposures occurred every other day during a week. HDI metabolites in urine samples were hydrolyzed to HDA with sodium hydroxide for

detection and analysis. Due to the high reactivity of HDI, free HDI was not expected to be found in urine or plasma. Among the three volunteers, the average urinary excretion of HDA was 39% (range: 9 to 94%) of the estimated inhaled dose, and the average half-time for urinary excretion was 2.5 hrs (range: 1.0 to 4.3 hrs). No free HDA was found in urine samples taken before exposures or in unhydrolyzed urine sampled during and after exposure, indicating that HDA in urine was covalently bound. Also, no HDA could be found in hydrolyzed plasma samples (detection limit: <0.1 µg/l).

In another metabolism study, five subjects exposed to 25 µg/m^3 (3.6 ppb) HDI for 7.5 hrs resulted in an average excretion of HDA in hydrolyzed urine samples of 16% (range: 11 to 21%) of the estimated inhaled dose (Brorson *et al.*, 1990). The average half-time for urinary excretion of HDI metabolites was 1.1 to 1.4 hrs; greater than 90% of the urinary elimination was completed within 4 hrs after termination of exposure.

Male workers (n=19) in HDI monomer production or manufacturing were monitored with personal air samplers for HDI exposure over an 8-hr work shift, followed by analysis for HDA in hydrolyzed urine samples (Maitre *et al.*, 1996a). The mean ± standard deviation (SD) HDI air concentration over the work shift was 14.3 ± 26.0 µg/m^3 (2.1 ± 3.8 ppb) and ranged from 0.30 to 97.7 µg/m^3 (0.04 to 14.2 ppb). The mean ± SD HDA in hydrolyzed urine was 8.52 ± 7.46 µg/g creatinine with a range of 0.30 to 27.70 µg/g creatinine. The urinary HDA was linearly correlated to the inhaled HDI concentration ($r = 0.6981$; $p = 0.001$). The initial half-life of urinary HDA was 1.5 hrs, although some amounts of urinary HDA were still detected 15-20 hrs after a single exposure. This finding suggested to the authors that an accumulation of HDI conjugates in the workers occurred during the work week.

Gaines *et al*. (2010) observed that both dermal and inhalation exposures to monomeric HDI in car painters were significant predictors of HDA levels in hydrolyzed urine samples when used as single exposure variables in multiple linear regression analysis. A tape-strip method was used to assess dermal exposure. No attempt was made to assess exposure to prepolymers in the car painters. The results also indicated biphasic elimination kinetics with a fast phase of 2.9 hrs. The half-life of the slow phase was not estimated. The authors proposed that the fast elimination phase likely reflects direct clearance from the plasma and correlates to relatively recent, within-day exposure, whereas the slower phase reflects urinary elimination of degradation products of HDI-adducted blood proteins such as albumin and hemoglobin.

Dosimetry models were developed to examine the differences in respiratory tract absorption of inhaled monomeric HDI vapor between rats and humans (Schroeter *et al.*, 2013). Nasal uptake efficiencies of 90-100% were measured in the rat. In comparison, nasal uptake in the nasal breathing human model was 78%. For lung dosimetry

simulations, an inhalation concentration of 1 ppb HDI was used with nasal breathing at twice minute volume. Due to high absorption in the upper respiratory tract, the average tracheal airway concentration was <0.04 ppb in the rat at the end of inhalation. In the human lung model, the average tracheal concentration of inhaled HDI at the end of inhalation was 0.16 ppb. However, HDI airway concentrations decreased rapidly in both models in the upper- and mid-bronchial airways with ongoing absorption/deposition at these sites. The predicted concentration was <0.02 ppb in the 10^{th} airway generation at the end of inhalation. Typically, the trachea is airway generation 0 and each airway branching increases the airway generation by one, ending with the fully alveolated airways as high as generation 23. The predicted total respiratory tract uptake of inhaled HDI vapor under nasal breathing was 99% in the rat and 97% in the human model. Oral breathing resulted in higher predicted tracheal HDI concentration in the human model (0.6 ppb). Despite the higher tracheal concentration, HDI did not penetrate much deeper into the lung. HDI concentrations in distal alveolar airways were $<2.0 \times 10^{-6}$ ppb, indicating virtually no HDI vapor reaches the deeper pulmonary airways of the human lung. Total respiratory uptake of inhaled HDI in the oral breathing model was estimated to be 87%. This was in the range of oral breathing measurements (61-90%) of respiratory retention of HDI vapor from volunteers exposed to concentrations of 5-15 ppb via a mouthpiece (Monso *et al.*, 2000).

Wall mass flux contour plots show high absorption rates of inhaled HDI in the anterior regions of the rat and human nasal passages, with greater fluxes in the extreme anterior region of the rat nose as compared to the human (Schroeter *et al.*, 2013). The cross-sectional average of wall mass flux in this anterior region was approximately 33 pg/cm^2-sec in rat vs. 12 pg/cm^2-sec in human. Due to higher tracheal concentrations in humans, wall mass flux values were greater in human airway generation 0-5 (approximately 0.8-0.9 pg/cm^2-sec) compared to the rat (approximately 0.3-0.4 pg/cm^2-sec). This finding supported the authors' conclusion that HDI exposure predominantly affects the nasal airway in rats and the bronchial airways in humans.

HDI Polyisocyanate Metabolism Studies

Liu *et al*. (2004) exposed 23 healthy auto body shop workers by mouthpiece to HDI biuret aerosol for two hrs to assess the concentration of HDA in hydrolyzed urine samples. All workers had previous workplace exposure to HDI biuret. The geometric mean equivalent HDI monomer exposure concentration was 23.5 ppb (162 µg/m^3) with a range of 17.1 to 29.7 ppb, as measured by a colorimetric paper tape method, which measures total HDI in equivalent monomer concentration in ppb, but does not differentiate monomer from oligomers. The geometric mean TWA concentrations for the monomer and oligomers were 53.8 and 98.7µg/m^3, respectively, when measured by a treated filter method. This method contains a filter that traps the aerosol allowing the

vapor to pass through, which is then collected on a glass filter fiber impregnated with 9-N-methyl-amino-anthracene (MAMA). The reaction with MAMA forms a stable, detectable derivative. Expressed as total reactive isocyanate group (TRIG) mass in unit volume of air, the concentration was 58.2 µg NCO/m^3. Post-exposure net increases in HDA urine concentrations varied 200-fold, from 0.4 to 101 µg/g creatinine with an average of 16.2 µg/g creatinine. The geometric mean half-life for urinary elimination was 2.8 hrs.

Using the treated-filter method, Liu *et al*. (2004) observed that urinary HDA levels did not correlate well with oligomer or TRIG exposure levels, but correlated better (albeit still not statistically significant) with HDI monomer-equivalent exposure levels (r_p = 0.45, p = 0.18) as measured by the tape method. The high variability in urinary HDA could not be explained by subject characteristics such as FEV_1, job category, age, and years working in the auto body industry. The authors speculated that respiration rate, HDI uptake, absorption, metabolism, or HDA-conjugate clearance could all be factors in the variability. The authors concluded that the correlation between inhalation exposure to HDI biuret and HDA was weak, indicating that metabolites other than HDA in hydrolyzed urine might better reflect the exposure, uptake, absorption, and clearance of oligomeric HDI.

5. Acute Toxicity of Hexamethylene Diisocyanate

5.1 Acute Toxicity to Adult Humans

Peer-reviewed studies of acute toxic endpoints in humans exposed to controlled levels of HDI are lacking. Therefore, a general presentation of the known acute effects from exposure to diisocyanates and polyisocyanates is summarized. Acute exposure to HDI monomer and polyisocyanates may irritate the mucous membranes in the respiratory tract (i.e., nose, throat and lungs) with symptoms of runny nose, sore throat, coughing, chest discomfort and breathing difficulty (Covestro LLC, 2015a). Eye irritation can also occur with symptoms of reddening, itching and swelling. Acute exposure in individuals with preexisting, nonspecific bronchial hyperreactivity may induce an asthmatic attack or asthma-like symptoms. Exposure to high levels may lead to bronchitis, bronchial spasm and pulmonary edema. Acute high exposure can also cause reactive airways dysfunction syndrome (RADS), an asthma-like illness developing after a single exposure to a high concentration of an irritating substance that persist for months or years (Redlich and Karol, 2002).

In an unpublished acute study, SIDS (2001) briefly summarizes the results of three male volunteers exposed to a series of increasing concentrations of HDI. No odor was detected at 1 ppb (7 µg/m^3). One of three men was said to smell HDI at 5 ppb (35

µg/m^3), while all three could smell HDI at 10 ppb (70 µg/m^3). At 20 ppb (140 µg/m^3) HDI was clearly perceptible and caused slight irritation in two of three volunteers. Exposure to 100 ppb (700 µg/m^3) resulted in an acrid odor and clear irritation of eyes and throat. SIDS (2001) notes that this study was insufficiently reported.

High acute exposure of workers to HDI polyisocyanates and their thermal breakdown products have been documented (Franklin *et al.*, 2000). A total of eight specialty painters at three different power plants complained of dyspnea (i.e., difficult or labored breathing) following the painting of the surfaces of hot boilers that were not allowed to cool sufficiently prior to painting. Four of the eight workers also complained of rash. Upon examination, three workers had a positive methacholine challenge and two were borderline. Methacholine challenge testing is a method of assessing airway hyperresponsiveness. Polyisocyanates in the paint consisted of 3% HDI and 97% HDI prepolymers. Respiratory protection had not been used by the painters. The authors noted that the surfaces of the boilers were above 340ºF, the temperature at which HDI biuret dissociates to form volatile monomeric HDI and other toxic compounds. At follow-up four years later, five painters still had to use inhalation medication and one had progressive asthma and dermatitis. No attempt was made by the authors to simulate the exposure conditions experienced by the painters to estimate concentrations of HDI and other toxic thermal breakdown products.

Only two peer-reviewed studies are available in which healthy subjects were exposed to controlled levels of HDI monomer and prepolymers. However, acute health effects were not the main focus of these studies.

Five healthy, non-atopic males were chamber-exposed to an average monomeric HDI concentration of 25 to 29 µg/m^3 (3.6 to 4.2 ppb) for 7.5 hrs (Brorson *et al.*, 1990). HDI exposure had no effect on vital capacity (VC) or the forced expiratory volume in one second (FEV_1), and none of the subjects showed signs of bronchial hyperreactivity. Methacholine challenge did not affect spirometry or bronchial reactivity immediately after exposure or at 15 hrs post-exposure. No specific IgE and IgG antibodies against HDI or prepolymerized HDI were detected before or after provocation. Subjective measures of sensory irritation and odor were not part of the methods protocol in this study, although the authors did note a general lack of symptoms in exposed subjects. The authors concluded from their findings that the HDI exposure “had not harmed the mucous membranes in the respiratory system of the subjects to any serious extent”.

In a metabolism study (outlined in detail above in Section 4) by Liu *et al*. (2004), 23 healthy auto body shop workers were exposed by mouthpiece to HDI biuret aerosol for two hours to assess the concentration of HDA in hydrolyzed urine samples. All workers had previous exposure to HDI biuret. The geometric mean TWA concentrations for the

monomer and oligomers were 53.8 µg/m^3 (7.8 ppb) and 98.7µg/m^3, respectively. When expressed as total reactive isocyanate group (NCO) mass in unit volume of air, the concentration was 58.2 µg/m^3 for combined monomer and oligomers. Spirometry was performed several times during exposure. Exposure was ended if a 15% drop in FEV_1 from baseline was recorded. It was reported that none of the workers experienced an acute change in FEV_1 during exposure. No other details were provided.

5.2 Acute Toxicity to Infants and Children

No studies were found that observed acute toxicity of HDI monomer and polyisocyanates in infants or children.

5.3 Acute Toxicity to Experimental Animals

HDI Monomer and Polyisocyanate Lethality Studies

The 4-hour LC_{50} (lethal concentration for 50% of an animal test population) of monomeric HDI vapor in Wistar rats was 124 mg/m^3 (18 ppm) with an extrapolated LC_{01} of 70 mg/m^3 (10 ppm) (Pauluhn, 2000). Mortality rates of male and female rats were similar, so data were combined for LC estimates. Alternatively, the 4-hour LC_{50} for HDI isocyanurate respirable aerosol in the same strain of rats was found to be 462 mg/m^3, with an extrapolated LC_{01} of 163 mg/m^3. Nasal discharge, labored respiration, respiratory distress and blepharospasm (spasm of the eyelid muscle due to painful stimuli resulting in eyelid closure) were common signs in the rats for both HDI monomer and isocyanurate prepolymer. However, the relative higher potency, longer-lasting signs of respiratory distress and a more delayed onset of mortality of HDI vapor were indicative of respiratory airway irritation. Alternatively, grayish, somewhat distended lungs and edema in rats exposed to isocyanurate suggest the alveolar region was predominantly involved. In a lethality study with the biuret prepolymer, the 4-hour LC_{50} in male Swiss-Webster mice recorded 24 hrs postexposure was 91.2 mg/m^3 (Weyel *et al.*, 1982). Lung weights in exposed mice were elevated by 50% at 45 mg/m^3 and lethality began to occur at a concentration inducing a 20% decrease in respiratory rate (approx. 40 mg/m^3).

HDI Monomer Acute Toxicity Studies

Changes in minute volume and tidal volume were examined in Wistar rats with 30 min exposures to HDI monomer at 4, 10, 27, 112 and 158 mg/m^3 (0.6, 1.5, 4, 16 and 23 ppm) (Pauluhn, 2015). An immediate concentration-dependent reduction in minute volume was observed at all exposure concentrations. From this data, the author predicted the maximum depression in minute volume occurred at concentrations exceeding 70 mg/m^3 (10 ppm) for 30 min. Decreased tidal volumes occurred at lower

concentrations with a reversal when the concentration was increased to around 112 mg/m^3 (16 ppm). This change in tidal volume was suggestive of vapor saturation where the aerosol phase HDI begins reaching the lower airways. Aerosol phase HDI reaching the pulmonary region was verified with analysis of BALF in additional rats exposed to 112 and 190 mg/m^3 HDI for 30 min. Increased protein and PMN in BALF was observed at the highest concentration. Increased γ-glutamyl transpeptidase, a marker for lower airway exposure and injury, was observed in BALF at both 112 and 190 mg/m^3.

Sangha *et al*. (1981) determined the RD_{50} (concentration resulting in a 50% depression of respiratory rate) for monomeric HDI in Swiss-Webster mice, which is designed to evaluate sensory irritation to the upper respiratory tract. The 60 min and 180 min RD_{50}s were 0.35 and 0.17 ppm (2.4 and 1.2 mg/m^3), respectively. TDI was also assessed by the authors for respiratory depression and was observed to have a similar potency compared to HDI.

To compare the toxic effects of HDI monomer and HDI biuret, Lee *et al*. (2003) exposed C57BL/6 mice to 0.36 ppm (2.5 mg/m^3) HDI monomer vapor for 3 hrs, and separate groups of C57BL/6 mice to 1 or 10 mg/m^3 HDI biuret aerosol for 5 hrs. In contrast to exposure to HDI biuret, no cell influx of macrophages and neutrophils or lung pathology was observed in the mice exposed to the monomer. Lavage fluid protein peaked at a later time point compared to mice exposed to HDI biuret (18 hr vs. 72 hr) and the magnitude of the increase was much smaller. The authors concluded that HDI vapor primarily reacted in the upper airways and did not penetrate significantly into the lung, whereas HDI biuret preferentially deposited in terminal bronchioles and alveolar ducts. In addition, the authors suggested that mice may be more sensitive to HDI-biuret than rats since significant effects were observed in mice at 1 mg/m^3, whereas HDI-biuret-exposed rats in the 3-week exposure study by Pauluhn and Mohr (2001) observed NOAEL of 3 mg/m^3.

No other peer-reviewed acute studies, which are defined here as exposures of two-weeks or less, could be located in the literature for HDI monomer. However, a three-week intermittent exposure study in rats has been performed that included extensive histopathological examination of the respiratory tract (Sangha, 1984; Shiotsuka et al., 2006). This was the key study used for the derivation of an acute REL for HDI monomer. A summary of this study together with quantitative findings of HDI-induced nasal lesions is presented in Section 6.3.1.

HDI Polyisocyanate Acute Toxicity Studies

Pauluhn (2000) examined the pulmonary responses, concentration dependence and time course of pulmonary response markers in bronchoalveolar lavage fluid (BALF) of

female Wistar rats (30/dose group) following nose-only 6-hr exposure to 0, 3.9, 15.9, 54.3, or 118.1 mg/m^3 respirable HDI isocyanurate aerosol. Irregular and labored breathing were observed at 54.3 mg/m^3 and above. Significantly increased lung wet weights (p<0.05) were observed at 15.9 mg/m^3 and above. Pulmonary response markers in BALF included angiotensin-converting enzyme (ACE), protein levels, alkaline phosphatase, lactate dehydrogenase (LDH), glutathione and phosphatidylcholine, which were assayed at 0 and 3 hours, and at 1, 3, and 7 days following exposure. The most sensitive response was a concentration dependent increase of ACE and total protein in BALF, which peaked in parallel on postexposure day 1. These changes in BALF likely result from epithelial barrier dysfunction and/or impairment of the vascular endothelium. The percent relative change of ACE and total protein compared to control on postexposure Day 1 was about 8500 and 4500, respectively, at the high dose. At the lowest dose of 3.9 mg/m^3, ACE and total protein were both statistically significantly elevated (p<0.01) at 3 hrs postexposure. Total protein was still elevated 1 day postexposure (p<0.01). Both BALF markers had returned to control levels in all exposed groups by 3 days postexposure.

In the same study, significantly increased activity of LDH in BALF (p<0.05), a marker of cytotoxicity, was observed at 54.3 mg/m^3 and above (Pauluhn, 2000). Alkaline phosphatase levels in BALF, a marker of pneumocyte type II activity and/or toxicity, were maximally elevated about 3-fold above control values on day 1, but no concentration-dependent increase was apparent above 15.9 mg/m^3. Glutathione levels measured in BALF peaked on day 1 following exposure and returned to control levels by day seven. However, GSH levels in lung tissue decreased immediately after exposure followed by apparent rapid replenishment and remained elevated through day seven.

A linear log-concentration-log effect relationship was observed by Pauluhn (2000) for total protein in BALF (% relative change to controls vs. concentration, y = 1.6 + 0.94x), resulting in a NOAEL of about 3 mg/m^3. Based on the variability among control animals (mean + 2 SD equals 100-125%), the author stated that this value is consistent with a 6-hour NOAEL in the range of 2.6 to 3.5 mg/m^3. The author also concluded that isocyanurate concentrations of 15.9 mg/m^3 or less result in noncytotoxic pulmonary capillary barrier dysfunction (i.e., increased ACE and total protein in BALF). Above this concentration, pulmonary cytotoxicity is observed (i.e., increased LDH in BALF). The author further concluded that pulmonary levels of GSH may be a modulating factor of susceptibility; if the delivery rate of isocyanurate exceeds the replenishment rate of endogenous scavengers such as GSH, pulmonary injury is likely to occur.

Pauluhn (2002) also conducted an acute study of the relative potency of HDI isocyanurate and PMDI aerosols to produce early changes in BALF. Groups of female

Wistar rats were exposed nose-only to either isocyanurate (3.9, 15.9, 54.3 or 118.1 mg/m^3) or PMDI (0.7, 2.4, 8, or 20 mg/m^3) for six hours and BALF collected for analysis at 0 and 3 hours, and 1, 3 and 7 days postexposure. MMAD and GSD were in the range of 1.4-2.0 and 1.4-2.1 µm, respectively, for the aerosols. For both ACE and total protein in BALF, a clear concentration-effect relationship was observed for both aerosols at 3 hr and 1 day postexposure. For LDH in BALF, in terms of concentration dependence and magnitude of changes at 3 hrs and 1 day post-exposure, a similar relationship was not observed. When double-logarithmic representations of ACE and total protein were extrapolated to the level of the controls, the NOEL was described by the author as 0.5 mg/m^3 for PMDI and 3 mg/m^3 for isocyanurate. However, isocyanurate exhibited a steeper concentration-effect relationship compared to PMDI. The author concluded that differences due to the slope rather than a shift in the concentration-effect curves were suggestive evidence that the differences in NCO content of the aerosols (isocyanurate 22%, PMDI 31%) are not the cause of potency differences in the lung effects. The more lipophilic and electrophilic aromatic PMDI may reach susceptible cell membranes more readily, while the aliphatic isocyanurate has more time to react with endogenous nucleophiles contained in the lung lining fluid of the airways.

Using similar methodology, Pauluhn (2004) compared the relative acute pulmonary irritant potencies among a number of polyisocyanate aerosols, including HDI polyisocyanate mixtures, isocyanurate, uretdione (also known as uretidone) and asymmetric HDI-homopolymer. Concentration dependence and duration of effects for endpoints, including lung wet weight and BALF levels of total protein and LDH, were measured in male Wistar rats following nose-only 6-hr exposures to the polyisocyanate aerosols (Table 2). The mass median aerodynamic diameter (MMAD) for the substances in Table 2 ranged from 1.2 to 2.3 µm, with a geometric standard deviation (GSD) that ranged from ± 1.1 to 1.8 µm. The aerosols displayed similar concentration dependence and time course for the endpoints. Total protein in BALF peaked on Day 1 postexposure and resolved by Day 3 or 7 postexposure. LDH in BALF usually showed a similar trend. Among the HDI prepolymers and polyisocyanate mixtures, the irritant threshold concentration (defined as the point of intercept of controls (=100%) + 2 SD of pooled control data) for total protein in BALF was 2.7 to 4.1 mg/m^3 (Table 2). Three of the test substances included concentrations in which both a NOAEL and LOAEL were obtained for increased total protein. The HDI homopolymer (asymmetric) NOAEL and LOAEL were 0.45 and 3.29 mg/m^3, respectively, the uretdione NOAEL and LOAEL were 0.56 and 2.8 mg/m^3, respectively, and the HDI-based polyisocyanate (hydrophilic ester) NOAEL and LOAEL were 0.56 and 3.21 mg/m^3, respectively. Other endpoints, including lung wet weight, LDH in BALF and clinical observation of respiratory tract irritation, generally had higher NOAELs compared to total protein in BALF. In contrast

to the aerosols, the one semi-volatile diisocyanate (isophorone diisocyanate) displayed increasing total protein and LDH in BALF up to day 7 post-exposure, which is characteristic of upper airway irritation predominating over pulmonary irritation.

Table 2. NOAELs and LOAELs in mg/m^3 for various pulmonary endpoints in rats exposed acutely to HDI prepolymer and polyisocyanate aerosols (Pauluhn, 2004)

Test Substance	NOAEL Endpoints			Respiratory Tract Irritation	
	Lung Weights[a]	Protein[a]	LDH[a]	NOAEL[b]	LOAEL[b]
HDI Homopolymer (asymmetric)	1.3	2.7	29	3.3	15
Uretdione dimer	17	3.1	4.2	2.8	19
Isocyanurate	24	3.5	21	15.9	54
HDI-based polyisocyanate	27	4.1	20	3.1	18
HDI-based polyisocyanate (hydrophilic ester)	12	3.4	10	3.2	16

[a] Calculations by Pauluhn (2004) based on the point of intercept of controls (=100%) + 2SD of pooled data

[b] Based on clinical findings by Pauluhn (2004) suggestive of respiratory tract irritation

Irritant threshold concentrations were determined for two other HDI-based polyisocyanates, polymeric emulsifier modified polyisocyanate (prepolymer I) and oligomeric allophanic modified polyisocyanate (prepolymer II), based on the dose-dependent increase of protein in BALF (Ma-Hock *et al.*, 2007). The main reactive component of each formulation was isocyanurate with a free NCO content of 16.9-19% and a residual HDI monomer content of ≤0.13%. The MMAD (± GSD) ranges for the two substances were 1.7-2.6 µm (± 1.7-2.0 µm). Groups of male Wistar rats were exposed to the aerosolized formulations (target means of 0.5, 3, 15 (both formulations) and 50 mg/m^3 (formulation II only)) for six hrs followed by collection of BALF at 1, 3 and 7 days postexposure. Increased BALF protein one day post-exposure was the most sensitive indicator of irritation. Using a standard NOAEL/LOAEL approach and actual measured means, the NOAEL and LOAEL for increased total protein was 0.5 and 2.7 mg/m^3, respectively, for prepolymer I. For prepolymer II, the NOAEL and LOAEL for increased total protein was 3.0 and 16.7 mg/m^3. Using the threshold effect protocol established by Pauluhn (the intercept of the concentration-effect curve with a line parallel to the x-axis at y = 1 + 2 times the standard deviation, which defines the upper bound of normal variability), modeled NOAELs of 1.1 and 2.3 mg/m^3 were determined for prepolymer I and II, respectively. These were considered by the authors to be similar to threshold results observed by Pauluhn (2004) for other HDI-based isocyanates.

Pauluhn (2008b) also conducted a comparative study of the pulmonary effects in C57BL6J mice and Wistar rats following nose-only 6-hour exposure to 10 mg/m^3 HDI biuret containing either 0.1% or 2% HDI monomer. The MMAD for the aerosols

generated was 1.8-1.9 µm (GSD = 1.6). For most of the endpoints examined, including body weight loss, lung wet weight, changes in BALF markers (total cell count, LDH, total protein, and γ-glutamyl transferase), and foamy appearance of cells, rats were more responsive to the toxic effects of biuret than mice. Clinical observations following exposure also noted irregular breathing patterns, piloerection, and reduced motility in rats, while no adverse clinical observations were noted in mice. Residual content of HDI monomer (0.1% vs. 2%) in the two biuret aerosols produced no differences on BALF endpoints in either rats or mice.

In a two week exposure study, male Wistar rats (20 per group) were exposed to 1, 1.2, 4.6, 16.3, and 69.2 mg/m^3 HDI isocyanurate 6 hr/day, 5 days/week to examine the asthmogenic potential of the prepolymer (Pauluhn and Mohr, 2001). The MMAD ± GSD range of the aerosols generated were 1.2-1.7 µm ± 1.3-1.7, respectively. At the end of the exposure period, body weights were significantly decreased and alveolar macrophages in BALF were significantly increased (p<0.05) at 69.2 mg/m^3. Lung wet weights were mildly increased at this exposure level compared to controls and did not reach statistical significance at p<0.05. Other markers of respiratory tract inflammation in BALF (lactate dehydrogenase, alkaline phosphatase, protein, γ-glutamyl transpeptidase, PMN) were unchanged at all concentrations. Lung function measurements did not reveal any increase in nonspecific bronchial hyperreactivity to aerosolized acetylcholine. Other lung function and blood gas measurements did not provide conclusive evidence of a deterioration of lung function other than a minimal decrease in tissue recoil at the two highest concentrations as a result of fluid accumulation. Histopathological effects were not found in the upper or lower respiratory tract. The authors noted that the rats were euthanized 4–5 days after the last exposure, so some recovery may have occurred. Markers of inflammatory changes in BALF, such as increased total protein, are usually at normal levels by 3 days following acute exposure to HDI isocyanurate. The authors suggest that reversibility of these changes occurs as rapidly following 2-week intermittent exposure as it does following a single acute exposure.

The effect of 3-hr exposures to inhaled HDI biuret on respiratory rate was investigated in Swiss-Webster mice (Weyel *et al.*, 1982). Exposure concentrations ranged from 25 to 131 mg/m^3. The MMAD and GSD for the polyisocyanate aerosol generated were 0.6 µm and 2.4, respectively. Mice initially showed a decrease in respiratory rate with a pattern similar to sensory irritation, which later changed to a pattern similar to pulmonary irritation that included a pause between each breath. Recovery was slow to nonexistent, up to 30 min or more postexposure at the highest concentrations. Decreased respiratory rate in tracheally cannulated mice did not begin until 60 to 120 min into exposure, and with a respiratory pattern entirely due to pulmonary irritation.

Thus, the authors concluded HDI biuret has an effect on respiratory rate due to irritation of both the upper and lower respiratory tract.

Lee *et al*. (2003) chamber-exposed groups of C57BL/6 mice to 1 or 10 mg/m^3 HDI biuret aerosol for 5 hr and assessed the pulmonary effects up to 2 weeks following exposure. The MMAD of the aerosol was 0.81 and 1.96 µm for the low and high dose groups, respectively (with a GSD of about 1.2 for aerosols generated in both exposure groups). A dose-dependent decrease in breathing frequency and an increased enhanced pause (Penh), that was maximal at end of exposure, were observed. The authors indicated these respiratory effects are characteristic of pulmonary irritants, probably caused by reflex bronchoconstriction or an induced breathing pattern change. At both exposure concentrations, lavage fluid protein and neutrophil influx occurred following exposure, while total cells and macrophages increased after an initial decrease immediately after exposure. Proliferative lesions identified histologically were maximal 90 hrs after exposure in mice exposed to 1 and 10 mg/m^3. Dose-dependent bromodeoxyuridine incorporation in alveolar ducts and in terminal bronchiole epithelial cells, an indicator of cellular proliferation, was also maximal 90 hrs after exposure and was statistically significantly increased (p<0.05) in both 1 and 10 mg/m^3 exposure groups.

The effect of 3-hr exposures to inhaled HDI biuret (0, 8, 18, 28, 33, 79, and 121 mg/m^3) on respiratory rate was also investigated in groups of English short-haired guinea pigs (Ferguson *et al.*, 1987). The ventilatory response to 10% CO_2 was used to evaluate the pulmonary toxicity of HDI biuret. Biuret exposures resulted in coughing in all exposed animals and apnea at higher concentrations. Concentration-dependent increases in respiratory rate and decrease in tidal volume were observed. The ventilatory response to 10% CO_2 was much greater than that obtained during air breathing and was characteristic of a lung restriction response (failure to increase tidal volume, but with an increase in respiratory frequency above the normal increase seen during CO_2 challenge). Histopathology of lungs in guinea pigs exposed to 121 mg/m^3 biuret revealed extensive microscopic inflammatory changes but no frank edema. Based on previous work in mice by Weyel *et al*. (1982), Ferguson and associates observed that guinea pigs are more resistant to pulmonary edema induced by acute exposure to biuret. Repeated 3 hr exposures to biuret (27.5 to 34.4 mg/m^3) up to 11 days resulted in an adaptation to the ventilatory response to CO_2, as opposed to a cumulative effect demonstrated for TDI from a previous study by Sangha and Alarie (1979).

Table 3 below provides a summary of the studies presented in Section 5.3.

Table 3. Summary of acute and subacute inhalation exposure studies in animals for HDI monomer and polyisocyanates.

Reference	Species	Exposure	Results
HDI Monomer and Polyisocyanate Lethality Studies			
Pauluhn (2000)	8-week old Wistar rats, males and females combined	Nose-only 4 hrs HDI – approx. 100 to 140 mg/m^3 HDI isocyanurate – approx. 200 to 1100 mg/m^3	· HDI LC_{50}: 124 mg/m^3 (18 ppm) · HDI isocyanurate LC_{50}: 462 mg/m^3
Weyel` *et al*., (1982)	Young adult male Swiss-Webster mice	Chamber exposed 4 hrs HDI biuret 25 to 131 mg/m^3	· LC_{50} – 91.2 mg/m^3
HDI Monomer Acute Toxicity Studies			
Pauluhn, 2015	Wistar rats, age and sex not identified	Nose-only 30 min HDI monomer 4, 10, 27, 112 or 158 mg/m^3	· Maximum minute volume depression above 70 mg/m^3 · ↓ tidal volume reversed at or above 110 mg/m^3 suggesting vapor saturation and aerosol phase HDI reaching pulmonary region · ↑ total protein, PMN at 112 mg/m^3 and above, and ↑ γ-glutamyl transpeptidase at 158 mg/m^3
Sangha *et al*. (1981)	Young adult male Swiss Webster mice	Chamber exposure to HDI monomer: 0.128, 0.290 or 1.950 ppm for 60 min; 0.062, 0.128 or 1.690 ppm for 180 min	· 60 min RD_{50} 0.35 ppm (2.4 mg/m^3) · 180 min RD_{50} 0.17 ppm (1.2 mg/m^3)
Lee *et al*. (2003)	Male C57BL/6 mice 2 to 6 mo of age	HDI monomer: nose-only 3 hrs to 2.5 mg/m^3 (0.36 ppm)	· No neutrophil or macrophage influx, no lung pathology. Total protein in BALF peaked at later time point compared to HDI biuret exposures.
Pauluhn (2000)	8-week old female Wistar rats	Nose-only 6 hrs HDI isocyanurate 0, 3.9, 15.9, 54.3, or 118.1 mg/m^3	· ↑ ACE and total protein in BALF at 3.9 mg/m^3 - most sensitive irritant response. · ↑ lung wet weight at 15.9 mg/m^3 · ↑ LDH in BALF at 54.3 mg/m^3
Pauluhn (2002)	8-week old female Wistar rats	Nose-only 6 hrs HDI isocyanurate: 0, 3.9, 15.9, 54.3 or 118.1 mg/m^3 PMDI: 0, 0.7, 2.4, 8, or 20 mg/m^3	· ACE and total protein in BALF showed clear concentration-effect relationship for both isocyanates. · NOEL based on above endpoints: 3 mg/m^3 for HDI isocyanurate 0.5 mg/m^3 for PMDI · NCO content not reason for isocyanate potency differences

Table 3. Summary of acute and subacute inhalation exposure studies in animals for HDI monomer and polyisocyanates (continued)

Reference	Species	Exposure	Results
HDI Polyisocyanate Acute Toxicity Studies			
Pauluhn (2004)	Young adult male Wistar rats	Nose-only 6 hrs HDI isocyanurate, uretidone, asymmetric HDI-homopolymer, or HDI polyisocyanate mixtures	· ↑ total protein in BALF ranged from 2.7 to 4.1 mg/m^3 for HDI polyisocyanates. Based on benchmark dose approach (point of intercept of controls (=100%) ± 2 SD) for total protein in BALF.
Ma-Hock *et al*. (2007)	8-week old male Wistar rats	Nose-only 6 hrs 2 HDI-based prepolymers (mainly isocyanurate) 0, 0.5, 3, or 15 mg/m^3	· ↑ total protein in BALF at 1.1 to 2.6 mg/m^3 using benchmark dose approach with intercept of conc.-effect curve y = 1 + 2 SD
Pauluhn (2008b)	Young adult male C57BL6J mice and male Wistar rats	Nose-only 6 hrs 10 mg/m^3 HDI biuret with 0.1 or 2% HDI monomer	· Rats more sensitive than mice to most endpoints (e.g., weight loss, ↑ lung weight, ↑ LDH, total protein and total cell count in BALF. · Residual content of HDI monomer (0.1 vs. 2%) showed no differences in toxic endpoints in both species.
Pauluhn and Mohr (2001)	8-week old male and female Wistar rats	Nose-only 6 hr/day, 5 days/wk for 2 wks HDI isocyanurate 1, 1.2, 4.6, 16.3, and 69.2 mg/m^3	· ↓ body weight at 69.2 mg/m^3 · No change in lung wet weight · ↑ macrophages in BALF at 69.2 mg/m^3 · No change in total protein or other markers of inflammation in BALF · No change in lung function with exposure to acetylcholine · Recovery time from either acute or 2-week exposure appears similar
Weyel` *et al*., (1982)	Young adult male Swiss-Webster mice	Chamber exposure 4 hrs HDI biuret 25 to 131 mg/m^3	· Lung weight increased by 50% at 45 mg/m^3 · Respiratory effects indicate irritation of both upper and lower airways: initial ↓ in respiratory rate, followed later by pause between breaths · Cannulated mice showed ↓ respiratory rate only after 60-120 min of exposure

Table 3. Summary of acute and subacute inhalation exposure studies in animals for HDI monomer and polyisocyanates (continued)

Reference	Species	Exposure	Results
HDI Polyisocyanate Acute Toxicity Studies (continued)			
Lee *et al*. (2003)	Male C57BL/6 mice 2 to 6 mo of age	HDI biuret: Chamber-exposed 5 hrs 0, 1 or 10 mg/m^3	· Dose-dependent ↑ enhanced pause (Penh) · Dose-dependent ↑ BrdU label in alveolar ducts and terminal bronchioles · At both concentrations - ↑ total protein, neutrophils and macrophages in BALF, and ↑ lung lesions
Ferguson *et al*. (1987)	Adult male English short-haired guinea pigs	3 hr chamber exposure HDI biuret 0, 8, 18, 28, 33, 79 and 121 mg/m^3	· Coughing and apnea at higher concentrations · Subsequent exposure to 10% CO_2 suggestive of lung restriction response to HDI biuret · Only microscopic inflammatory effects in lungs at 121 mg/m^3
	Adult male English short-haired guinea pigs	3 hr exposures for 11 days HDI biuret 27.5 - 34.4 mg/m^3	· Adaptation to ventilatory response to CO_2

6. Chronic Toxicity of Hexamethylene Diisocyanate

6.1 Chronic Toxicity to Adult Humans

6.1.1 Background

Repeated exposure to HDI monomer and polyisocyanates, usually on the order of months to years, may result in a process by which the immune system will produce antibodies in response to the isocyanate exposures. This process where the respiratory system becomes sensitive and subsequently allergic to isocyanates is called sensitization. Subsequent exposures, usually well below worker exposure limits, may then result in an asthmatic reaction (Covestro LLC, 2015a). Symptoms following exposure of sensitized individuals include chest tightness, wheezing, cough, and shortness of breath, which are temporally related to the exposure. It is unclear why only a small proportion (5-15%) of exposed workers develop isocyanate-induced asthma, although exposure characteristics and certain host factors are undoubtedly involved (Redlich and Karol, 2002). An asthmatic reaction resulting from HDI polyisocyanate exposure may be “early” (<1 hr after exposure), “late” (occurring 2-4 hr later), or “dual”. Isolated early reactions are not common in isocyanate asthma.

The pathogenesis of isocyanate-induced asthma is a complex process and still largely unknown (Redlich *et al.*, 2007). In addition, the clinical presentation of isocyanate-induced asthma is variable, often complicating its recognition and diagnosis. However, the clinical manifestations and pathophysiological changes observed have similarities to those in atopic, or 'allergic', asthma, including airway hyperreactivity, the presence of eosinophilic lung infiltrates, and mucus hypersecretion in airways (Del Prete *et al.*, 1993; Herrick *et al.*, 2003). Allergen-specific IgE is a mediator of many of the symptoms of bronchial hyperreactivity in atopic asthma, but is not found in most individuals with isocyanate-induced asthma. Isocyanate-specific IgE antibodies are found in only 5-30% of patients with isocyanate-induced asthma, and even occasionally found in exposed workers who are asymptomatic. A similar low percentage of HDI polyisocyanate-specific IgE is found among spray painters with asthma induced by HDI polyisocyanates (Campo *et al.*, 2007). The titers of isocyanate-specific IgE are usually low compared with titers typically reported for high molecular weight antigens such as pollen and dust mite antigens (Redlich and Karol, 2002).

Isocyanate-specific IgG, including HDI polyisocyanate-specific IgG, is detected in a greater percentage of exposed workers (22-43%) than those with isocyanate-specific IgE (Campo *et al.*, 2007; Wisnewski *et al.*, 2012). In most cases, isocyanate-specific IgG has generally been found to correlate with exposure of an individual to isocyanates but does not correlate with atopy or asthma. HDI-specific IgG has been proposed as a biomarker in workers to complement isocyanate exposure monitoring (Wisnewski *et al.*, 2012).

Similar to workers with TDI and MDI-induced asthma, airway histology and immunohistochemistry in workers with HDI-induced asthma revealed an increased number of total inflammatory cells, basement membrane thickening, and increased numbers of total and activated (CD25+) T cells (Redlich *et al.*, 1997). Unlike atopic asthma, which is usually a TH_2 T-cell response, isocyanate-induced asthma presents more of a mixed Th_1/TH_2 T-cell response with more prominent airway neutrophilia and interleukin-8 (Lemiere *et al.*, 2002; Redlich *et al.*, 2007). T cell clones from workers with isocyanate-induced asthma show secretion of high levels of both IFN-γ and IL-5, but not IL-4. T cell clones from atopic asthmatics are primarily CD4+ and produce IL-4 and IL-5, but no IFN-γ.

Piirila *et al*. (2000) conducted a long-term follow-up (mean: 10 yrs) of 245 workers that had been diagnosed with occupational asthma due to HDI, TDI, MDI, or related polyisocyanates. Ninety-six of the 245 asthma cases (39%) were a result of exposure to HDI polyisocyanates. Some workers (15%) reported occasional isocyanate exposure in their current work. Overall, 82% of the workers still experienced symptoms of asthma, 34% used no medication and 35% were on regular medication, suggesting to

the authors that there is a generally poor prognosis for those with isocyanate-induced asthma. Clinical re-examination of 91 workers indicated that FEV_1 reduction did not exceed the predicted decline over time in either smoking or nonsmoking patients.

A favorable prognosis for isocyanate respiratory sensitization is more likely for those diagnosed with better lung function, a milder degree of bronchial hyperreactivity, an early reaction (as opposed to a late reaction), and shorter duration of symptoms (Ott *et al.*, 2003). Therefore, it is important that once diisocyanate-related asthma develops, further exposures should be fully avoided.

Chronic overexposure may also cause lung damage resulting in an accelerated loss of lung function that is unrelated to sensitization. This is possibly a result of recurrent exposures to brief irritant levels of HDI polyisocyanates over time. Both sensitization and lung function decrements may be permanent. In rare cases, life-threatening hypersensitivity pneumonitis (also known as extrinsic allergic alveolitis) has been documented in spray painters using HDI polyisocyanate-based paints (Usui *et al.*, 1992; Bieler *et al.*, 2011). Hypersensitivity pneumonitis is a restrictive disease affecting the bronchioles and alveoli, whereas asthma is an obstructive respiratory disease usually affecting the bronchi.

6.1.2 Diagnosis of HDI Polyisocyanate-Induced Asthma

A clear diagnosis of isocyanate-induced asthma requires that the patient is shown to have asthma, that the patient's asthma is temporally related to exposure, and that the asthma is linked to exposure to a specific agent (Banks *et al.*, 1996). The diagnosis of asthma has to be established, either by demonstrating reversible airflow obstruction, or the presence of nonspecific bronchial hyperresponsiveness (NSBHR) with challenge exposure to methacholine or histamine (Redlich and Karol, 2002). Isocyanate asthma without NSBHR is uncommon in symptomatic patients who are still exposed to isocyanates. The use of inhalation challenge tests to sub-irritant levels of the polyisocyanate thought to cause asthma is considered the most reliable and straightforward method for establishing a diagnosis (Vandenplas and Malo, 1997). For workers exposed to HDI polyisocyanates and showing signs of isocyanate-induced asthma, the commercial product used at their work is nebulized for exposure in chambers, or via face mask or mouthpiece. Closed circuit exposure devices described by Vandenplas and Malo (1997) make it possible to generate steady concentrations of HDI aerosol.

However, inhalation challenge testing is neither 100% sensitive nor specific. Baur *et al*. (1994) conducted challenge tests in 42 HDI-polyisocyanate-exposed workers complaining of work-related cough, chest tightness, and/or dyspnea. The challenge

tests in chambers usually started with 5 ppb for 15 min followed by 10 ppb for 30 min, and finally 20 ppb for 5 min. A positive result was identified as an increase in specific airway resistance (sRaw) of 100%, and obtaining values of at least 2.0 kilopascals × sec. Using this protocol, only 7% (3 of 42) of the workers had a positive reaction to HDI polyisocyanate. Similar chamber exposure studies with TDI and MDI in groups of workers with symptoms due to these diisocyanates resulted in 30% (12 of 40) and 36% (21 of 59) positive reaction, respectively. The authors suggested reasons for the negative chamber results, particularly for HDI, include exposures not being high enough or long enough to induce asthma, concomitant exposure to other irritants in the workplace, workers still in a preclinical stage of sensitization, workers mistaking mild respiratory irritation for dyspnea, and decreased isocyanate hypersensitivity resulting from leaving the workplace months before chamber exposure occurred. Indeed, Sastre *et al*. (2003) has shown that some workers suspected to have HDI-induced asthma did not show an asthmatic reaction after the first inhalation challenge with HDI, but did result in NSBHR (>20% fall in FEV_1) with challenge to methacholine that was not present before the HDI exposures. In addition, a second challenge with HDI in some of these non-responding workers resulted in an asthmatic reaction.

Cross-reactivity between different isocyanates may occur (Baur *et al*. (1994). Alternatively, exclusive hypersensitivity to a specific isocyanate prepolymer has been demonstrated. Vandenplas *et al*. (1993) exposed 20 spray painters with possible occupational asthma due to HDI-based spray paints to pure HDI and HDI prepolymers (i.e., the paint hardener to which the subject had been exposed at work) on separate days. Mean concentrations of HDI and prepolymer were mostly kept between 10 to 20 ppb. A reduction in FEV_1 >20% due to exposure was considered a positive reaction. Under these conditions, ten of the subjects had a positive reaction. Among these subjects, five had a positive reaction to both HDI monomer and prepolymers, and another four had a positive reaction only to the prepolymers. The last subject had a positive reaction to HDI monomer but not the prepolymers. Exposure concentrations were similar, so the authors concluded that differences in concentration between HDI and prepolymers were not a factor in the response. Also, retesting of some positive subjects to HDI in aerosol form did not change the response, suggesting the physical state of the monomer was not a factor in the response to the monomer. The authors concluded that different responses to HDI monomer and prepolymers were due to a difference in bronchial reactivity to the two forms of HDI.

HDI polyisocyanate formulations consist mainly of a mixture of several different HDI-based prepolymers, solvents, and only a fraction of monomeric HDI. Innocenti *et al*. (1986) performed a specific challenge test on a car painter suspected to have HDI-induced occupational asthma. Exposure to HDI-based polyisocyanate paint

(concentration not recorded) resulted in a late asthmatic reaction. However, exposure to TDI (2-3 ppb) and the HDI paint solvents (xylene, toluene, methyl ethyl ketone, ethyl- and iso-butylacetate) on separate days did not produce an asthmatic response. These findings suggest that cross-reactivity between different isocyanates does not occur in all sensitized workers, and that solvents in HDI-based polyisocyanate paints are likely not responsible for asthmatic responses.

6.1.3 Measurement of Airborne HDI Polyisocyanates

In order to determine the concentration of a specific isocyanate in air, appropriate sample collection and handling, derivatization, separation, identification, and quantification methods must be followed (NIOSH, 1998; Streicher *et al.*, 2000). The efficiency and applicability of a given collection method are influenced by factors such as the expected isocyanate state (e.g. aerosol versus vapor) and the type of sampling (e.g. personal versus area) being done. Sample collection usually involves an impinger containing a solvent, a sorption tube containing adsorbent, a denuder, and/or a filter. Given that the isocyanate group is reactive, the sample is often exposed to a derivatization agent during or soon after collection. Derivatization limits isocyanate loss due to side reactions (e.g., with water to produce diamines), reduces interference by other molecules in the collected sample, and thus improves the selectivity and sensitivity of the method. The derivatization agent may be contained within an impinger or impregnated into a filter for immediate derivatization of the sampled isocyanates, or added later to a collected sample. To ensure derivatization of isocyanate compounds specifically, some *a priori* knowledge is required regarding the compounds likely to be collected and their respective reactivity to the derivatization agent. The appropriate derivatization agent will react with a specific region (functional group) of the isocyanate molecules contained in the sample to create derivatives.

In general, NIOSH Method 5525 may offer the most specificity, sensitivity, and applicability (NIOSH, 1998). Sample collection is achieved using a glass fiber filter impregnated with a derivatization agent, an impinger containing a derivatization agent, or a combination of the two. While the filter collects particles of all sizes, it most efficiently collects and derivatizes small particulates (≤2 μm). The impinger traps diisocyanate vapors and larger polyisocyanate particles in the aerosol. Use of the impinger in addition to the filter improves collection of larger particles which may not disperse on the filter to allow derivatization of the collected isocyanates. This method is appropriate for personal or area sampling, and the impinger can be used for collecting particles with short (<several minutes) or long half-lives (NIOSH, 1998). Alternatively, Pisaniello and Muriale (1989) used two impingers in series and observed collection efficiency for HDI polyisocyanate vapor and aerosol to be better than 97%.

After the sample has been derivatized, its components are separated for identification of individual compounds within the sample (NIOSH, 1998). This is most often accomplished by reversed-phase high-performance liquid chromatography (RP-HPLC). Quantification can then be achieved by creating a calibration curve using different standard concentrations. Because multiple chemicals can co-elute to produce identical/similar retention times, use of a selective detector (e.g. ultraviolet-visible or fluorescence), which responds only to specific classes of chemicals, can aid identification. Use of two different selective detectors in series can increase the selectivity and sensitivity of detection.

6.1.4 Principal Occupational Studies

The summarized studies in this section consist of cross-sectional and longitudinal occupational studies in which exposure to HDI monomer and/or HDI-based polyisocyanates predominated. In most cases, the studies conducted pulmonary function testing to look for pulmonary function changes associated with exposure. Longitudinal studies are the primary means for assessing asthma onset prevalence and changes in pulmonary function with time. However, most are cross-sectional studies, which are more practical to run but have disadvantages due to healthy worker effect or survivor bias as well as variable diagnostic criteria and poorly defined cohorts of workers (Redlich *et al.*, 2007). The first three studies examined workers exposed only to HDI monomer in HDI chemical manufacturing plants. The remainder, and majority, of the summarized studies examined spray painters exposed to HDI-based polyisocyanates. Presentation of the studies in Table summary format (Table 10) immediately follows the study summaries.

Diller et al. (1985)

This briefly reported German cross-sectional occupational study examined the lung function of 81 workers in a HDI monomer manufacturing plant (Diller *et al.*, 1985) (translated from German by OEHHA). The control cohort consisted of 86 workers without exposure to HDI or other lung irritants. Prior to 1970, HDI exposure at the plant was said to be sometimes unavoidable and resulted in at least 10 workers leaving due to respiratory problems between 1950 and 1970. Since 1970 when plant upgrades were installed, HDI exposure was described as rare, except for occasional spills and malfunctions. Ten air measurements taken in the plant in June-July 1982 showed no detectable HDI exposure (detection limit: 0.01 mg/m^3, or 1.5 ppb). A pipeline malfunction that occurred during the study resulted in release of HDI in the facility in which HDI was detected by the workers by its odor. The authors noted that detection of the odor indicates HDI levels possibly exceeding 20 ppb (138 $\mu g/m^3$), the MAK level

(German Maximale Arbeitsplatz-Konzentration, or maximum workplace concentration). The wearing of respirators was then required at this level.

Regardless of this malfunction, the plant reported that no worker had visited the medical department since 1970 due to HDI-related respiratory tract irritation. Lung function tests (FEV_1, FVC and R_{aw}) indicated that the HDI group had better lung function than the control group (Table 4). The authors suggested poorer lung function in the control group may be due to higher relative body weight (102.4% vs. 104.7% in controls), slightly longer tenure (13.0 vs. 16.3 yrs in controls) and greater percentage of smokers (57% vs. 71% in controls) in the control group compared to the HDI group. A "survivor population" effect was discounted by the authors due to the low turnover rate, 8.1-8.2% per year from 1973 to 1982 for both exposure groups. The study conclusion was that no deterioration in lung function is observed with substantial compliance to the upper exposure limit of 20 ppb (138 µg/m^3) HDI.

Table 4. Lung function measurements in HDI monomer-exposed workers and control workers (Diller *et al.*, 1985)

%FEV_1		**%FVC**		**R_{aw} (cmH_2O/l/sec)**	
HDI (n = 81)	Controls (n = 86)	HDI (n = 81)	Controls (n = 86)	HDI (n = 81)	Controls (n = 86)
96.0 ± 1.19*	87.4 ± 2.19	105.5 ± 2.28	100.0 ± 2.75	2.81 ± 0.10	2.84 ± 0.12

* Lung function values are mean ± standard error

Hathaway et al. (1999)

The pulmonary function of 32 workers in an isocyanurate and biuret production facility was evaluated during a nine-year (1988-1997) longitudinal study to determine if exposure to HDI causes an accelerated decline in FEV_1 and FVC (Hathaway *et al.*, 1999). The average exposure duration was 8.4 yrs among cases, and pulmonary function tests were conducted yearly. Average age for the matched pairs was 41.7 yrs for cases and 39.8 yrs for controls. The workers were compared to matched controls, which consisted of 32 workers within the same facility, but were not involved in HDI prepolymer production. Monitoring results were only for monomeric HDI vapor, which is used to produce the prepolymers, because the handling procedures of biuret and isocyanurate in the facility did not present a significant inhalation exposure. The TWA exposure to HDI for the workers was 0.5 ppb (3.4 µg/m^3) while in the unit (roughly 2 hrs per day), and approximately 0.13 ppb (0.89 µg/m^3) as an 8-hour TWA. The average daily (4-minute) peak exposure of 2.9 ppb (19.9 µg/m^3), and the average daily 15-minute short-term exposure was 1.5 ppb (10.3 µg/m^3). These exposures were for time periods when respiratory protection was not used.

The pulmonary function results for the nine-year exposure period is shown in Table 5. No statistically significant differences (two-tailed t-test for paired comparisons with 95% confidence intervals) were seen between cases and controls, although an effect of smoking status on lung function was observed.

Table 5. Annual average change in FEV_1 and FVC (in liters) by smoking status (Hathaway *et al.*, 1999)

Smoking Status	FEV_1		FVC	
	Cases	Controls	Cases	Controls
Never (n=12)	-0.022	-0.023	-0.013	-0.003
Former (n=9)	-0.044	-0.053	-0.022	-0.044
Current (n=11)	-0.067	-0.051	-0.043	-0.033
All Subgroups (n=32)	-0.044	-0.041	-0.026	-0.025

The authors noted the statistical power to detect changes was low due to the small number of individuals in the study, but this was countered somewhat by the long follow-up period. The authors concluded that the results suggest no adverse effects on FEV_1 or FVC occurs with TWA daily exposures of 0.5 ppb (3.4 µg/m^3) HDI, and occasional peak exposure in the range of 1 to 10 ppb (6.9 to 69 µg/m^3) HDI.

Cassidy et al. (2010)

A retrospective study expanded upon the work by Hathaway *et al*. (1999), including workers at an additional HDI monomer/prepolymer manufacturing plant, and a longer duration of follow-up yielding a larger cohort of workers (Cassidy *et al.*, 2010). The exposed workers (n=100) were matched against a control group of workers from other sections of the plants with no history of diisocyanate exposure. The average duration of observation was 13.5 yrs for Plant 1 exposed workers (n=57) and 9.9 yrs for Plant 2 exposed workers (n=43). Minimum employment to participate in the study was two years. Medical histories were conducted annually, with pulmonary function tests conducted at least once per year. It was reported that the workforce was fairly stable with no one leaving employment due to work-related respiratory problems. TWA 8-hour exposure to HDI monomer was assessed only when workers were not wearing respiratory protection. A total of 236 airborne HDI samples from Plant 1 were included for analysis. HDI concentrations ranged from nondetectable (88 total nondetects) to 31 ppb (213 µg/m^3). The mean concentration was 0.78 ppb (5.37 µg/m^3) with four samples above 10 ppb (69 µg/m^3). Plant 2 had a total of 29 samples which ranged from

nondetectable (6 total nondetects) to 2 ppb (14 µg/m^3) with a mean level of 0.3 ppb (2.1 µg/m^3).

During the study, no cohort members developed respiratory sensitization or any other work-related respiratory problems. For pulmonary function test analysis, FEV_1 and FVC were significantly different (p<0.001) for the group as a whole and for the ever-smoked subgroup, indicating that pulmonary function decreased faster in controls in these respective groups compared to that of the exposed group over time (Table 6).

Table 6. Annual average change in FEV_1 and FVC (in liters) by smoking status (Cassidy *et al.*, 2010)

Smoking Status	FEV_1		FVC	
	Cases	Controls	Cases	Controls
Never smoked (n=48)[a]	-0.019	-0.030	-0.020	-0.031
Ever smoked (n=52)[a]	-0.028	-0.050[b]	-0.026	-0.047[b]
All Subgroups (n=100)[c]	-0.023	-0.041[b]	-0.024	-0.040[b]

[a] n for exposed group only. Controls were matched by smoking status but the specific n was not presented.
[b] P<0.001, compared to case (exposed) group
[c] n=100 for both case and control groups

The greater decline in pulmonary function values for the controls was described as an unexpected result. The authors ruled out as confounders differences in excess weight gain, exposure to other isocyanates, and a pulmonary function test learning effect from newer employees. Exposure of control workers to other chemical agents was presented and discussed, but was ultimately considered an unlikely factor for the lower pulmonary function values. This included some Plant 2 control workers in the Rare Earths unit exposed to high particulate levels (mean: 6.2 mg/m^3) during the first seven years of the study. Although not addressed by the authors, this difference could be a result of a “healthy worker effect”, in which only healthy people went to work in the isocyanate units. the study noted that the decline in FEV_1 for all subgroups combined was within the range of annual FEV_1 declines reported in other studies.

An estimate of pack-years smoked to determine the amount of smoking between control and exposed groups had not been evaluated. The accelerated decline in pulmonary function among smoking controls compared to the smoking exposed group strongly suggested to the authors that the controls were heavier smokers. In conclusion, they determined that this study provides support for the current ACGIH TLV-TWA of 5 ppb (34 µg/m^3).

Grammer et al. (1988)

Grammer *et al*. (1988) conducted an 18-month prospective study in 150 motor vehicle paint sprayers exposed to monomeric HDI and isocyanurate for incidence of immunologic sensitization and work-related respiratory symptoms. The workers were divided into seven groups on the basis of job category. The mean exposure duration of all workers combined was 36 months, but ranged from 24 to 51 months depending on job category. Mean age of all workers was 44 ± 7 yrs. Forty-one air samples were collected from work areas at two times during the study. The measured air concentrations were adjusted for those job categories in which workers were required to wear respirators (e.g., a protection factor of 100 for paint sprayer jobs; a protection level of 10 for plumbers). Only isocyanurate exposure levels were used for statistical purposes because this was the major HDI oligomer to which workers were exposed. Mean exposure levels ranged from 5.3 $\mu g/m^3$ for spray paint repairers to 75 $\mu g/m^3$ for paint mixers. Eighteen workers reported at least one respiratory symptom on their questionnaire, but most of these workers had some other unrelated respiratory diagnosis.

IgE and IgG antibodies against HDI and isocyanurate, both conjugated to human serum albumin (HSA), were determined using enzyme-linked immunosorbent assay (ELISA). During the study only one worker developed work-related respiratory disease (chest tightness and cough) with exposure to HDI oligomers. The symptoms disappeared when the worker moved to another location in the facility. However, this worker had no antibody against HDI-HSA, isocyanurate-HSA, or HSA. The percent of all workers with indices >2.0 (optical density of worker serum / optical density of control sera as measured by enzyme-linked immunosorbent assay) for IgG to isocyanurate-HSA and HDI-HSA and for IgE to isocyanurate-HSA and HDI-HSA was 12%, 13%, 4%, and 5%, respectively. The percent of workers with positive indices for IgG or IgE to HSA was 0% and 0.7%. Although 21% of the workers had a positive antibody result, which was generally low-level IgG, no correlation was found between antibody level and exposure duration. The authors concluded that the HDI oligomer levels may be too low in this study to induce immunologically mediated respiratory disease, but were in the range for development or presence of low-level IgG antibody formation.

Alexandersson et al. (1987)

Alexandersson *et al*. (1987) examined the lung function of car painters in a cross-sectional study. The subjects included 41 car painters, 48 car platers (exposed to the same solvents and grinding dust as car painters, but not to isocyanates) and 70 car mechanics (not exposed to the chemicals of car painters or platers). The car painters/platers were matched with the mechanic control group. Average employment

was seven years in all three groups. The paint hardener used by the painters contained 40-50% HDI biuret and 0.5-1% monomeric HDI. Lung function tests for painters were conducted on Monday morning prior to work and on Friday afternoon. The mean exposure of the painters was 115 µg/m^3 biuret and 1.0 µg/m^3 (0.15 ppb) HDI monomer. The exposures had been adjusted by a protection factor based on the type of respiratory protection used. Painters were estimated to have an active spraying time that was 23% of the total working period, and the exposures involved a number of peaks during work. Short-term peaks up to 13,500 µg/m^3 biuret were recorded.

Subjective symptoms, including eye, nose and throat irritation, were elevated in painters compared to mechanic controls (p=0.14), and significantly increased in platers (p<0.05). The use of breathing masks by painters was thought to be a factor for reduced irritation compared to platers. Spirometry measurements including FEV_1, FVC, and MMF (maximum mean expiratory flow in L/sec) were not different in painters compared to mechanics. However, nitrogen washout on Monday morning showed increases in closing volume in relation to percent vital capacity (VC%) in the painters vs. the controls (p=0.003). Nitrogen washout is a test for measuring anatomic dead space in the lung during a respiratory cycle. The closing volume is the amount of air remaining in the lungs when the flow from the lower sections of the lungs becomes severely reduced or halts altogether during expiration as the small airways begin to close. The authors stated the increase in closing volume is suggestive of “small airways disease”. The mean value for VC% increased during the workweek, but the increase was not significant (p=0.17, binary test). Dividing the lung physiology findings into smokers and nonsmokers did not change the results. With the exception of some subjective symptoms, car platers were no different from the mechanic control group. The authors noted that the effects of exposure may be underestimated, as no attempt was made to evaluate any painters that may have left the job due to lung function impairment resulting from exposure.

Tornling et al. (1990)

Tornling *et al*. (1990) conducted a six-year longitudinal study in a subgroup of Swedish car painters from the initial study by Alexandersson *et al*. (1987) to evaluate possible lung function changes related to exposure. The studied group consisted of 36 car painters and 115 controls (mainly car platers and car mechanics) with pulmonary function tests conducted in 1978 and again in 1984. The car painters’ mean employment time was 16.5 yrs with a minimum of one year at the beginning of the study. Mean age in 1984 was 39.8 yrs for painters and 38.4 yrs for controls. Exposures during daily work operations were estimated from 98 air samples, both inside and outside the respirators, for analysis of monomeric HDI and HDI biuret. Eighteen air samples with sampling times of less than 3 min were also collected to determine peak

exposure levels exceeding the Swedish Short Term Exposure Level, 0.07 mg/m^3 (0.010 ppm) for HDI and 0.20 mg/m^3 for HDI biuret, for 5 min exposures during specific high exposure situations. Individual exposure was calculated from workplace data and proportion of work tasks, and accounted for car painters' work habits and type of respirator used. The TWA exposure for car painters was 1.5 µg/m^3 (0.2 ppb) monomeric HDI and 90 µg/m^3 biuret. Peak exposure levels were exceeded frequently by most car painters when sufficient respiratory protection was not used.

A statistically significant increase in wheezing was observed among never smoking car painters (p<0.01) and current/ex-smoker painters (p<0.05) compared to their respective controls. Symptoms from airways and eyes were also greater in car painters, but did not reach statistical significance. One car painter developed IgE-mediated isocyanate asthma during the study and terminated his job employment. However, none of the other car painters showed increased IgE compared to controls. Smoking/ex-smoking car painters had a significantly larger decrease in FEV_1, FVC and VC compared with smoking/ex-smoker controls (Table 7). No significant differences were observed in any nitrogen washout variables, including VC%, phase III, as observed earlier by Alexandersson *et al*. (1987). No differences in lung function were seen between non-smoking car painters and non-smoking controls.

Table 7. Statistically significant changes in lung function (in liters) from 1978-1984 by smoking status (Tornling *et al.*, 1990)

Smoking Status	FEV_1		FVC		VC	
	Cases	Controls	Cases	Controls	Cases	Controls
Never	+0.07 (n=9)	-0.23 (n=27)	-0.10 (n=9)	-0.20 (n=27)	-0.13 (n=9)	-0.14 (n=27)
Former & Current	-0.37[a] (n=27)	-0.17 (n=115)	-0.57[c] (n=27)	-0.23 (n=115)	-0.46[b] (n=27)	-0.18 (n=115)

[a] p<0.05; [b] p<0.01; [c] p<0.001

Linear regression analysis found that a decrease in FVC correlated significantly (p<0.05) with the number of exposure peaks (5 min exposure above 0.07 mg/m^3 for HDI and 0.20 mg/m^3 for biuret), but not for mean exposure levels. The authors speculated that the lung function decrements in smokers and ex-smokers exposed to HDI and biuret were due to a lowered defense system of the lungs, and that the group of nonsmokers was possibly too small to make any conclusions. Dahlqvist *et al*. (1995) later noted that current smokers had a significantly higher yearly number of peak exposures compared to never smokers. They opined that this may indicate that car painters who smoke are less likely to use respiratory protection in exposure situations, and thus show a greater decline in lung function.

Dahlqvist et al. (1995)

Dahlqvist *et al*. (1995) reanalyzed the car painter data from Alexandersson *et al*. (1987) and Tornling *et al*. (1990) and found that a decrease in lung function within the week may be a marker of vulnerability in those workers that showed long-term decrements in lung function. Alexandersson *et al*. (1987) had previously found a slight, but non-significant, average decrease in FVC and FEV_1 in the car painters between Monday morning prior to work and on Friday afternoon after a working week. A significant correlation (p=0.009) was then found by Dahlqvist *et al*. between the decline in FVC within the week and the long term (six year) decline in FVC. This finding was based on a subset of 20 car painters working the entire 1978-84 period and had three spirometric examinations. The workers were standardized for the effects of aging and smoking, and adjusted for number of peak exposures. The TWA exposures for these workers were 0.0014 mg/m^3 (0.20 ppb) HDI and 0.09 mg/m^3 HDI biuret. Median yearly number of peak exposures (>2.0 mg/m^3 for ≥30 sec and up to 3 min) to biuret without respiratory protection was 2000.

No significant correlation was found between the change in FVC during a shift and the long-term decline in FEV_1, or the change in FEV_1 within the week and the long-term decline in FVC and FEV_1. The normal circadian rhythm in FVC and FEV_1 will usually result in higher values in the afternoon compared with morning values. The authors suggested that the non-significant decrease in FVC and FEV_1 observed by Alexandersson *et al*. (1987) during a working week may be more important than first observed because the decline could have been partially overridden by the normal variation in lung function.

Akbar-Khanzadeh and Rivas (1996)

Both short- and long-term studies on spray paint workers exposed to HDI monomer and unspecified HDI polyisocyanates were conducted by Akbar-Khanzadeh and Rivas (1996) in a plant that encapsulated automobile glass (a process in which polymeric material is injected around a glass insert as it is suspended in a mold). In the short-term study, pulmonary function changes within and during the work week were measured in 17 urethane mold operators and were compared to a group of 20 nonexposed workers. The mean duration of exposure in the exposed workers was 9 months. Personal air sampling (n=6) recorded a mean of 10.7 µg/m^3 (1.6 ppb) HDI monomer and 90 µg/m^3 HDI polyisocyanate. No difference in respiratory symptoms was observed between the two groups. No reduction in pulmonary function (FEV_1, FVC, FEV_1%FVC) in exposed workers was observed during Monday or Friday work shifts, or across the workweek from Monday morning through Friday afternoon.

In the long-term study, workers were followed prospectively for 2.5 yrs (Oct. 1989 to March 1992) to assess changes in pulmonary function. The study consisted of three groups of workers: 65 painters and mold operators exposed to HDI polyisocyanates and VOCs, 40 solvent-exposed workers only exposed to VOCs, and 68 office workers with no chemical exposure. Mean age of the workers, depending on the subgroup, was 28 to 35 yrs. Minimum employment to be included in the study was one year. The TWA workplace exposure of painting and molding workers yielded 0.0010 ppm (0.007 mg/m^3) monomeric HDI (n=8) and 0.29 mg/m^3 HDI polyisocyanates (n=5). Some workers were also exposed to MDI prior to 1992, although the exposures were described as low. MDI mean exposure during molding operations was 0.45 ppb (4.6 µg/m^3) (n=7).

No differences in respiratory symptoms were found between the three groups, and no workers developed isocyanate-induced asthma during the study. On average, the percent change in FEV_1 from zero decreased significantly (p<0.001) in all HDI polyisocyanate-exposed worker subgroups stratified by smoking habit over the 2.5-year follow-up (Table 8). FVC also decreased significantly in all HDI polyisocyanate-exposed workers combined, and in the smoking subgroup. No statistically significant changes were observed in the solvent-only exposed group. In the controls, FEV_1%FVC decreased significantly in smokers and all controls combined.

Table 8. Percent change in pulmonary function during 2.5 Year follow-up (Akbar-Khanzadeh and Rivas, 1996)

Group	n	% Changes		
		∆FEV_1	∆FVC	∆FEV_1%FVC
Total				
HDI exposed	65	−2.8 ± 5.8[a]	−1.5 ± 5.6[b]	−1.2 ± 5.8
Solvent only	40	0.6 ± 7.1	1.2 ± 5.0	−0.8 ± 4.1
Control	68	−0.4 ± 6.4	1.1 ± 6.1	−1.2 ± 4.5[b]
Smoker				
HDI exposed	32	−2.8 ± 6.5[a]	−2.3 ± 6.4[b]	−0.5 ± 6.7
Solvent only	18	0.2 ± 8.2	0.6 ± 5.3	−0.7 ± 4.3
Control	32	−0.2 ± 6.1	1.9 ± 5.8	−1.9 ± 4.1[b]
Nonsmoker				
HDI exposed	33	−2.8 ± 5.2[a]	−0.7 ± 4.7	−1.9 ± 4.9[b]
Solvent only	22	0.9 ± 6.2	1.6 ± 4.8	−0.8 ± 4.0
Control	36	−0.6 ± 6.7	0.4 ± 6.4	−0.6 ± 4.7

[a] Significant difference from zero, p<0.001; [b] Significant difference from zero, p<0.05

When the magnitude of changes was compared using one-way analysis of variance, significant differences in FEV_1 and FVC were found among the groups. No difference in FEV_1 change was apparent between groups when stratified by smoking status, which was suggested to result from loss of study power. The modified Bonferroni multiple comparison test showed that changes in FEV_1 for the HDI polyisocyanate-exposed

workers was significantly greater (p<0.05) than that for the solvent-only exposed workers. This test also found that the changes in FVC for the HDI polyisocyanate-exposed workers were significantly greater than that of the control group. Stratification by smoking status showed significant differences between the HDI polyisocyanate-exposed workers and the controls in both smokers and nonsmokers with respect to FVC. The authors suggested that the pulmonary function deficits observed during the long-term study was due to lack of respiratory protection being used, while lack of pulmonary deficits in the short-term study was due to implementation of respiratory protection by the time this part of the study was conducted. The authors also noted that although exposure to VOCs-only did not cause pulmonary function deficits, they did not rule out the possibility that VOCs in combination with HDI isocyanates may cause pulmonary function deficits.

Randolph et al. (1997)

A cross-sectional study was conducted in South African spray painters to determine if cross-shift changes in pulmonary function occur (Randolph *et al.*, 1997). Forty spray painters were examined by questionnaire and pulmonary function tests, and an air sample of unspecified duration was taken from each of 40 spray booths the workers were using during a spraying operation. Spirometric readings were taken before and after each work shift, which involved a spraying operation lasting about 30 min. Baseline spirometric lung function results were generally slightly above predicted values. The mean duration that the subjects had worked as a spray painter was 11.9 years (range: 1 to 30 years). The mean HDI polyisocyanate concentration in the spray booths was 6.46 ± 6.64 mg/m^3 (mean ± SD). Forty percent of spray booths had ventilation standards below specified legislation and only 55% of spray painters were provided with regulation respiratory protective equipment, most of whom choose less protective equipment than the employer provided. Adjustment of the polyisocyanate concentration for respiratory protection used by the workers was not performed. Additionally, non-regulated spray painting (i.e., moonlighting) was conducted by many of the painters after hours.

Chronic respiratory symptoms were more common in ever smokers (n=27) than in nonsmokers (n=13), but the difference did not reach statistical significance. Ten percent of subjects had shortness of breath with wheezing. However, none of the spray painters were diagnosed with occupational asthma, which the authors noted could be a result of a “healthy worker” effect and a limitation of cross-sectional studies. Fifty-five percent of the spray painters complained of eye irritation or experienced a burning sensation of the eyes while spray painting. The authors attributed the eye symptoms to the lack of use of eye protection equipment by most of the workers. In addition, 32% claimed to have dermatitis of the hands.

A significant decrease (p=0.0002) in mean cross-shift FEV_1 of 130.5 ± 203.19 ml was observed in the spray painters. Ten subjects (25%) had a clinically significant cross-shift decrease in FEV_1 >250 ml. None of the potential determinants of FEV_1 examined (e.g., age, length of service, smoking status, etc.) were found to be statistically significant by multiple regression analysis, with only isocyanate index (concentration divided by the Oregon permissible exposure limit of 1 mg/m^3) approaching significance (p=0.082). The single measurement of polyisocyanate concentration from each spray booth was explained by the authors as a possible reason for lack of significance because the measurement may not have reflected a true 8-hour time-weighted average exposure. The authors cited substandard ventilation of the spray booths, lack of adequate personal respiratory protection, and poorly designed spray guns as likely reasons for decreased cross-shift FEV_1 in the spray painters.

Redlich et al. (2001)

A comprehensive cross-sectional epidemiologic study, the Study of Painters and Repairers of Autobodies by Yale (SPRAY), was initiated in 1997 (Redlich *et al.*, 2001). The purpose of the study was to elucidate isocyanate exposures and characterize the health effects of the exposures on actively employed auto body shop workers. The results were presented in several published reports. In the health effects portion of the SPRAY study, 75 auto body shop workers from 12 different shops were recruited and underwent a week-long physiologic evaluation, blood studies, and filled out worker diaries and questionnaires. The mean age of the workers was 39.1 yrs, with a median duration in the auto body industry of 15 yrs. Job categories included painter (n=18), technician (n=39) and office worker (n=18). Worker exposures were primarily to HDI biuret and HDI monomer. Fifty-three percent of workers had no exposures during the survey week, 23% had between 1 and 10 HDI peak exposures (associated with spraying, mixing and priming tasks), and 24% had $\geq$10 peak exposures. The median number of peak exposures was 34 for painters and 0 for technicians and office workers. The number of such peak exposures was significantly associated with job category (p<0.001 by Kruskal-Wallis test).

A low rate of overt asthma was found among the 75 workers. Only one had a current diagnosis of asthma and none was currently under treatment for asthma. Eleven had a $PD_{20} \leq 25$ mg and only two had a $PD_{20} < 8$ mg, the clinically used cut-off for a positive methacholine challenge test. The authors suggested the low rate of asthma could be due to not only a “healthy worker” effect, but also a “healthy shop” effect, whereby owners of cleaner shops are more likely to participate in surveys. Regarding the immunologic findings, HDI-specific IgG was present in 34% of HDI-exposed workers and HDI-specific IgE was detected in two workers. Lymphoproliferative responses to HDI were evaluated by determining the proliferative response of each subject’s

peripheral blood mononuclear cells to HDI-monomer-HSA conjugates *in vitro*. HDI-specific lymphocyte proliferation was present in 30% of HDI-exposed workers, and their proliferation index was mostly in the range of 2 or less. Limited data suggest a proliferation index >3 may indicate the presence of isocyanate asthma or some degree of immunologic sensitization to isocyanates. Since the median duration of employment was 15 yrs, the authors theorized that it is unlikely most of the subjects with a proliferation index around 2 or more would go on to develop isocyanate-induced asthma. HDI biuret-HSA and HDI monomer-HSA induced proliferative responses that were strongly correlated (Spearman r = 0.86; *P* = 0.0001), suggesting that the monomer and biuret forms of HDI are antigenically similar, both capable of inducing HDI-specific lymphocyte responses and IgG.

Job category was found to be associated with several parameters including symptoms of chest tightness and shortness of breath, cross-week change in FEV_1, methacholine responsiveness, and HDI-specific proliferation and HDI-specific IgG. However, because of small sample sizes none of these associations reached statistical significance. Greater number of peaks of unprotected HDI exposure per week was associated with greater cross-week decline in FEV_1 (p=0.067) and shortness of breath (p=0.005). The authors concluded that the presence of HDI-specific immune responses is found in a large proportion of healthy HDI-exposed workers without overt asthma. However, in order to identify suspected cases of HDI asthma in this cohort, the authors planned to perform long-term follow up and HDI-specific challenges and present the findings in a future report.

Redlich et al. (2002)

Redlich *et al*. (2002) conducted a one year follow-up at some of the original auto body shops investigated by the SPRAY group (Redlich *et al.*, 2002). Among 48 participants, 34 had stayed at the same shop, 11 had left their original shop, and three were lost to follow-up. No statistically significant changes in physiology, symptoms, and immunologic responses from baseline to follow-up were observed, although there was a trend towards a greater decrease in FEV_1 and FVC in painters and technicians compared to office workers. One worker with suggestive symptoms and physiology in the first study had become an overt case of asthma at the one year follow-up. This worker reported asthma-like symptoms and became more hyperresponsive to methacholine (PC_{20} = 5 mg/ml) at follow-up. The findings also suggested that a healthy worker effect may exist among auto body shop workers, in that workers who left their shop were more likely to have a history of asthma (23% vs. 3%, p<0.05), bronchial hyperresponsiveness (23% vs 9%), HDI-specific IgG (64% vs 29%; p<0.05) and HDI-specific proliferation (stimulation index 2.0 vs 1.3; p<0.05). Workers that left also tended to be younger and less experienced in the industry.

Glindmeyer et al. (2004)

In this cross-sectional study, 240 spray painters at four U.S. Air Force aircraft maintenance plants were examined for pulmonary function changes related to cumulative total exposure to HDI-based paints (Glindmeyer *et al.*, 2004). Mean age of the workers was 40.3 yrs, and the mean years of spray painting was 10.3 yrs. Exposure monitoring was conducted twice at each plant and consisted of personal sampling of overspray during painting. The exposure data appeared log-normally distributed, so geometric means were estimated as well as arithmetic means. Overall, the total aerosol personal exposure geometric mean was 7.3 mg/m^3 and the isocyanate group in total aerosol geometric mean was 0.8 mg/m^3. Exposure was also expressed as total respirable aerosol and isocyanate group in respirable aerosol. Geometric mean values for total respirable aerosol and isocyanate group in respirable aerosol were 0.8 and 0.08 mg/m^3, respectively. None of the exposure estimates were adjusted for use of respiratory protective equipment.

A later study of some of the same spray painting facilities used an analytic technique (Iso-Chek^{TM}) that measured both vapor and aerosol phase HDI polyisocyanates (Carlton and England, 2000). Mean task based and 8-hr TWA concentrations of HDI monomer for the spray painters were 2.4 and 0.1 ppb, respectively. Mean task based and 8-hr TWA concentrations of HDI oligomers (HDI biuret and/or isocyanurate) were 2.27 and 0.11 mg/m^3, respectively.

When expressed by cumulative respirable paint aerosol and isocyanate group, the authors noted that there appeared to be an association between cumulative exposure and both reduced lung function and increasing percentages of workers reporting symptoms (Table 9). In addition, increasing cumulative exposure appeared to be associated with elevated levels of COPD (data not shown).

Table 9. Lung function (in percent of predicted value ± standard error) and symptoms by cumulative respirable paint aerosol (Glindmeyer *et al.*, 2004)

Respirable aerosol level (mg/m^3-yrs)	N	Mean Age	FEV_1 %	FVC %	FEV_1/FVC %	FEF25-75 %	Shortness of breath & wheeze (%)
0.5 – 5.8	66	37.0	95.0±2.1	97.3±1.7	97.3±1.0	90.2±3.8	4.6
6.1 – 14.1	58	38.3	94.5±1.6	96.3±1.5	98.1±0.8	91.0±2.9	1.7
14.2 – 20.5	56	40.7	92.5±1.7	96.7±1.8	95.7±1.2	84.1±3.5	5.4
21.1 – 57.6	60	45.4	90.6±1.7	94.0±1.7	96.3±0.8	81.5±3.5	8.3

Multiple linear regression modeling with percent predicted lung function as an outcome variable was then conducted. Expressing the results as percent predicted lung function meant that neither age, sex, or race were statistically significant nor will they have an effect on the estimated regression coefficients for exposure, and were thus selected out

of the regression models. All four cumulative exposure indices (total and respirable paint aerosol, and estimated isocyanate group in total and respirable aerosols) were significantly associated (p<0.05) with decreased FEV_1, FEV_1/FVC and FEF_{25-75}. In addition, years spray painting was significantly associated with decreased FEV_1 and FEF_{25-75}. However, none of the regressions were significant for those having, versus those lacking, the symptom complex of shortness of breath with wheeze, which might indicate asthma. Finally, current use of respiratory protection was not found to be associated with lung function (e.g., lung function was not improved with greater use of respiratory protection). This finding suggested to the authors that recent or current practices of respiratory protection may not be sufficient in protecting workers.

The authors concluded that the adverse effects on lung function suggest current standards for spray painting may not be protective enough even though worker exposure was reduced by frequent use of respiratory protective equipment. They also noted that paint formulations are complex mixtures, and adverse effects may result from components other than isocyanates.

Pronk et al. (2007)

Pronk *et al*. (2007) assessed the prevalence of respiratory symptoms and sensitization in 581 workers in the spray-painting industry. Personal exposure was estimated by combining personal task-based inhalation exposure measurements and time-activity information. Exposure was expressed as estimated monthly cumulative personal exposure for total isocyanate group (NCO) concentration, including monomeric HDI, HDI-biuret, and HDI-isocyanurate. The median exposure among spray painters (n=241) was 3,682 µg $NCO \times m^3 \times hr \times mo^{-1}$. The average time worked as a painter was 14.9 years. "Other" workers (n=290) in the industry were exposed only to 8 µg $NCO \times m^3 \times hr \times mo^{-1}$, although they had worked a total of 3.4 yrs as a spray painter during a total employment time of 19.2 yrs. A third group were office workers (n=50) with no recent history of exposure. Respiratory protection equipment was mandatory for spray painters, but was not factored into the isocyanate exposure of the workers.

The prevalence of chronic obstructive pulmonary disease-like (COPD) symptoms (including chronic cough, chronic phlegm, and shortness of breath) and asthma-like symptoms (including wheezing and chest tightness) was greater (p<0.05) in spray painters compared to office workers. The prevalence of asthma-like symptoms in "other" workers was also greater compared to office workers. Significant log-linear associations (p<0.05) with exposure were found for asthma-like symptoms, COPD-like symptoms, work-related chest tightness, and work-related conjunctivitis. Individuals with asthma-like symptoms were more likely to have bronchial hyperresponsiveness, as assessed by methacholine challenge. These individuals also had lower baseline FEV_1,

FEV_1/FVC, and maximum mid-expiratory flow (MMEF) between 90 and 96% compared with symptom-free workers. However, no clear association could be found between work-related symptoms and lung function.

Overall, the authors concluded that despite a possible healthy worker effect, exposure–response relationships were demonstrated for respiratory symptoms and sensitization in a population of spray painters. Blood samples were also collected from the workers for serologic analysis of HDI-specific IgE and IgG antibodies. IgE and IgG antibodies were found in up to 4.2% and up to 50% of spray painters, respectively, depending on type of HDI-human serum albumin conjugates used. IgE was associated with exposure and work-related chest tightness, while IgG was strongly associated with exposure. The authors concluded that, at most, specific IgE plays a role in a minority of individuals with symptoms.

Dragos et al. (2009)

In this prospective study of 298 apprentice car painters in Quebec, Canada, the incidence of work-related respiratory symptoms was investigated in relation to changes in specific antibody levels over a period of up to 19 months (Dragos *et al.*, 2009). Participants were assessed at the beginning and end of the training program using questionnaires, methacholine challenges and measurements of HDI-specific IgE, IgG and IgG4. IgG4 is one of four subclasses of IgG in humans. HDI monomer and oligomers were surveyed in personal breathing zone (n = 51) and area air sampling (n = 41) during regular and specific spraying activities for periods varying from 5 to 120 min. When spraying, students were wearing masks but not always those recommended, and masks were often removed inappropriately to inspect work. Personal breathing zone levels of HDI monomer were low (median: 0.001 mg/m^3, range: 0.0006-0.006 mg/m^3) while HDI oligomer levels were high (median: 0.283, range: 0.033-0.916 mg/m^3). Area sampling levels were much lower (data not shown).

Thirteen subjects developed work-related lower respiratory symptoms of asthma diagnosis and/or bronchial hyperresponsiveness, and 19 developed work-related symptoms of rhinoconjunctivitis. Only one of the subjects had both workplace symptoms. Reporting a diagnosis of asthma and a positive methacholine challenge test (PC ≤ 16 mg/ml) were significantly associated with the incidence of work-related lower respiratory symptoms ($p = 0.01$). Associations with changes in immunoglobulins were observed at maximum values, the 95^{th} and 97^{th} percentile of the HDI-specific IgE and IgG distributions, respectively, suggesting a more reactive subpopulation of individuals within the cohort. However, the authors were only able to show that increases in HDI-specific IgG and IgG4 appear to have a protective effect on the incidence of work-related lower and upper respiratory symptoms, respectively. Conversely, higher levels

of IgG4 in some subjects at the beginning of the study, probably related to previous HDI isocyanate exposure or other personal factors, appear to be a risk factor leading to the subsequent development of work-related rhinoconjunctivitis. The authors concluded that assessment of specific antibodies to isocyanates may help identify subjects at risk of developing symptoms.

Pourabedian et al. (2010)

This study has some methodology limitations. However, the findings suggest that inadequate or no respiratory protection during spray painting operations results in decreased lung function following exposure.

A study was conducted in 43 Iranian automobile painters to determine if peak expiratory flow is affected by HDI exposure within and between working days (Pourabedian *et al.*, 2010). Each subject acted as his own control to obtain the daily variation in peak flow. The mean age of the workers was 37.9 yrs (range: 23 to 60 yrs) and mean work experience was 13.39 yrs (range: 3 to 30 yrs). Most workers were described as performing painting jobs one or two times a week with a mean daily exposure of 15 minutes. The authors did not differentiate between HDI monomer and polyisocyanates collected during spraying operations. However, glass fiber filters coated with a derivatizing agent were used to collect the air samples. Filters only collected particles, with small particulates (≤2 µm) collected most efficiently. The mean daily TWA HDI concentration in the breathing zone was 420$\pm$100 µg/m^3 (61$\pm$15 ppb). Mean weekly TWA HDI exposure was lower at 130$\pm$59 µg/m^3 (19$\pm$8.6 ppb) because workers performed spray painting tasks only 1-2 times per week.

On painting days, 40% of the workers showed a decrease of over 20% in peak flow over what would have been their normal peak flow. The mean peak flow on painting days was reduced from 537.8 L/min at the start of the shift to 479.1 L/min at the end of the shift. Peak flow was still reduced the following day at the start of the shift (518.9 L/min) and at the end of the shift (478.2 L/min). The difference between the two days was statistically significant (p=0.017). The authors used a mean weekly decrease in peak flow of ≥20% as a benchmark for significant respiratory impairment. This was observed in 39.5% of the workers (17 of 43 workers). HDI concentration and percentage decrease in mean peak flow were correlated (p<0.001) on the day of painting. In addition, a statistically significant correlation (p<0.001) in the percentage decrease in peak flow with age and work experience (i.e., the longer work experience, the greater percentage decrease in peak flow) was observed. No information was provided on respiratory signs or symptoms of the workers in this study. The authors did not describe whether respiratory protection was used, although they did note that inhalation exposure exceeded the TLV (5 ppb for HDI monomer vapor) by over ten-fold. Even

though exposure to organic solvents may have also occurred, the authors concluded the data strongly suggest that HDI affected peak flow in exposed workers.

Table 10. Summary of occupational studies in which workers were exposed to HDI monomer or HDI-based polyisocyanates

Study	Study type, Industry & Exposure	Findings
Diller *et al*., 1985	Cross-sectional study of 81 workers at HDI monomer plant Control cohort: 86 workers with no HDI or irritant exposure Facility said to be in compliance with 20 ppb exposure limit HDI concentration <1.5 ppb with 10 measurements	Compared to unmatched controls: · No lung function effects (FEV_1, FVC and R_{aw}) due to HDI exposure · Survivor population effect discounted due to low turnover, 8.1-8.2% per yr
Hathaway *et al*., 1999	9-yr longitudinal study of 32 workers at a HDI prepolymer production facility 8-hr TWA; 0.13 ppb HDI monomer; 0.5 ppb while in the unit (about 2 hrs/day) Occasional peak exposures: 1-10 ppb HDI monomer	Compared to matched controls: · No difference in lung function (FEV_1 and FVC) observed. · Smoking status effect on lung function was observed. · Statistical power was low for detection of HDI effects due to small n
Cassidy *et al*., 2010	19-yr retrospective study of 100 workers exposed to HDI monomer at two facilities that produce HDI and prepolymers. 8-hr TWA; 0.78 ppb HDI over 13.5 yrs at plant 1, and 0.3 ppb HDI at plant 2 for over 9.9 yrs.	· During the study, no work-related respiratory problems occurred compared to matched controls: · No lung function effects due to HDI · FEV_1 and FVC accelerated lung function decline in "ever smoked" control group · Greater decline in controls thought to be due to higher pack-yrs smoked
Grammer *et al*., 1988	18-mo prospective study in 150 paint sprayers Mean age: 44 yrs Mean exposure duration: 36 mo (range: 24-51 mo) Mean isocyanurate range for 7 job categories: 5.3-75 µg/m^3	· Most respiratory symptoms during study unrelated to HDI oligomer exposure. · One case of asthma-like disease which disappeared when removed from exposure. · 21% of workers positive antibody against HDI-HSA, mainly IgG; no correlation of antibody level with exposure duration.
Alexandersson *et al*., 1987	Cross-sectional study of 41 car painters, 48 car platers (no HDI exposure) and 70 car mechanics (no HDI or HDI-related solvent exposure) Mean employment: 7 yrs for all Mean age: 34 yrs (painters) and 31 yrs (platers) Mean exposure 115 µg/m^3 biuret and 1.0 µg/m^3 monomer	Matched against car mechanic controls: · Eye, nose and throat irritation increased in platers (p=0.05) but not painters. · No difference in FEV_1, FVC and MMF · Closing volume (%) increased in painters (p=0.003) Monday morning · No effect on lung physiology during workweek (Mon morning to Fri after work)

Table 10. Summary of occupational studies in which workers were exposed to HDI monomer or HDI-based polyisocyanates (continued)

Study	Study type, Industry & Exposure	Findings
Tornling *et al*., 1990	6-yr longitudinal study of car painters (n=36) and controls (n=115) Mean age at end of study: 39.8 yrs for painters and 38.4 yrs for controls Mean employment: 16.5 yrs Mean exposure 90 µg/m^3 biuret and 1.5 µg/m^3 monomer	· Increased wheezing in never smoking (p<0.01) and smoking/ex-smoking (p<0.05) painters · One painter developed IgE-mediated HDI asthma · Smoking/ex-smoking painters showed accelerated decline in FEV_1, FVC and VC compared to respective controls · Decreased FVC correlated with # peak exposures, but not for mean exposure
Dahlqvist *et al*., 1995	Additional analysis of Alexandersson *et al*. and Tornling *et al*. studies Subset of 20 painters working 1978-84. Mean age 41 yrs Employment duration: 25 yrs Mean exposure 90 µg/m^3 HDI biuret, 1.4 µg/m^3 monomer	· Significant correlation (p=0.009) between decline in FVC within week and 6-yr decline in FVC · Finding suggests that a subset of workers with short-term decrease in lung function is more vulnerable to long–term effects.
Akbar-Khanzadeh and Rivas, 1996	2.5-yr prospective study in paint and mold workers (n=65), solvent-only workers (n=40) and controls (n=68) Mean exposure 290 µg/m^3 HDI polyisocyanates and 1.0 ppb HDI Mean age ranges: 28-35 yrs Minimum exposure: 1 yr	Expressed as % change from zero: · FEV_1 decreased (p=0.001) in HDI-exposed smokers, nonsmokers and combined, and FVC decreased in HDI-exposed smokers and combined · FEV_1 decreased (p=0.05) in HDI workers compared to solvent workers · FVC decreased (p=0.05) in HDI workers compared to controls
Randolph *et al*., 1997	Cross-sectional study of spray painters (n=40) Mean employment: 11.9 yrs Mean age: 32 yrs Mean exposure 6.5 mg/m^3 HDI polyisocyanates	· 55% reported eye irritation · 32% reported dermatitis · Decreased (p=0.0002) cross-shift FEV_1 observed · Substandard ventilation, spray guns and personal protective equipment cited as reason for decreased within-shift FEV_1
Redlich *et al*., 2001	Cross-sectional study of auto shop workers: painters (n=18), technicians (n=39) and office workers (n=18) Mean employment: 15 yrs Mean age: 39.1 yrs Exposure as number of peak exposures to HDI monomer and HDI biuret	· Job category associated with chest tightness, shortness of breath, cross-week change in FEV_1, methacholine responsiveness, HDI-specific lymphocyte proliferation and IgG, but sample sizes too small to reach statistical significance · Greater number of peak exposures associated with greater cross-week FEV_1 decline and shortness of breath

Table 10. Summary of occupational studies in which workers were exposed to HDI monomer or HDI-based polyisocyanates (continued)

Study	Study type, Industry & Exposure	Findings
Redlich *et al*., 2002	1-yr follow-up of 48 auto shop workers from Redlich *et al*. (2001) 34 still at same shop 11 had left original shop 3 lost to follow-up Mean age: 43 yrs Mean employment: 20.5 yrs	· No significant change in respiratory symptoms at follow-up · One worker developed asthma · No significant decline in pulmonary function than expected in 1 yr · Detection of healthy worker effect: Workers that left more likely to have a history of asthma, bronchial hyper-responsiveness, and HDI-specific IgG and lymphocyte proliferation
Glindemeyer *et al*., 2004	Cross-sectional study of 240 spray painters Mean age: 40.3 yrs Mean employment: 10.3 yrs Task based total aerosol & total respirable aerosol isocyanate group geometric means: 0.8 and 0.08 mg/m^3, respectively	· Cumulative total and respirable paint aerosol (and isocyanate group total and respirable aerosol) in mg/m^3-yrs significantly associated (p<0.05) with decreased % predicted lung function (FEV_1, FEV_1/FVC and FEF_{25-75}) · Yrs spray painting significantly associated with decreased % FEV_1 and FEF_{25-75}.
Pronk *et al*., 2007	Cross-sectional study of 241 spray-painters exposed to HDI oligomers compared to office workers (n=50) Mean time worked: 14.9 yrs Mean age 37-39 yrs Median exposure: 3.68 mg NCO×m^3×hr×mo^{-1}	Compared to non-exposed office workers, (mean age 40 yrs): · Increased (p<0.05) COPD and asthma-like symptoms · Work-related chest tightness and conjunctivitis associated with exposure · Increased BHR to methacholine challenge in workers with asthma-like symptoms
Dragos *et al*., 2009	Prospective study up to 19 mo in 298 apprentice car painters. Median monomer levels 0.001 mg/m^3 and median HDI oligomer levels 0.283 mg/m^3 during spraying. Mean age: 23.5 yrs	· 13 subjects (4.4%) developed work-related lower respiratory symptoms (physician-diagnosed asthma and/or bronchial hyperresponsiveness). · 19 subjects (6.4%) developed work-related nasal symptoms. · Increased HDI-specific IgG and IgG4 inversely related to incidence of work-related lower and upper respiratory symptoms.

Table 10. Summary of occupational studies in which workers were exposed to HDI monomer or HDI-based polyisocyanates (continued)

Study	Study type, Industry & Exposure	Findings
Pourabedian *et al*. 2010	Within day and within week peak flow measured in 43 spray painters exposed to HDI aerosol. Mean age 37.9 yrs; mean work experience 13.39 yrs Daily and weekly TWA exposure was 420 and 130 µg/m^3, respectively.	· Peak flow reduced on painting days at end of shift, and still reduced the following day (p=0.017) · HDI concentration and % decrease in mean peak flow correlated on day of painting (p<0.001) · Mean weekly decrease in peak flow correlated with age and work experience (p<0.001)

6.2 Chronic Toxicity to Infants and Children

No studies were found reporting chronic toxicity of HDI monomer or HDI-based polyisocyanate exposure in infants and children. A study on the effects of occupational exposure to HDI polyisocyanates in adolescent spray painters has been published (Eifan *et al*., 2005). However, no information was provided in the study to suggest that adolescents are more sensitive to HDI polyisocyanates compared to adults.

In this study, pulmonary function, symptomology, and sensitivity to methacholine inhalation were examined in 72 apprentice adolescent (ages 15-20 yrs) car painters in Turkey (Eifan *et al.*, 2005). An age-matched control group consisted of 72 students from the same region with no known exposure to isocyanates. However, the car painting group had significantly heavier smokers than the control group. The mean age and working duration of the car painters were 17.47 yrs and 3.12 yrs, respectively. The average time the car painters worked per week was said to be 62.6 hrs. The type of HDI prepolymers sprayed was not identified in the study, and no attempt at quantifying exposure was done. Use of respiratory protection by the adolescents appeared to be inadequate or not used at all. The prevalence of current and ever wheezing was significantly higher (p<0.05) in car painters than in the control group, with a current nocturnal cough recorded as the most common complaint (p=0.015). Work-related symptoms, such as breathlessness, wheezing, chest tightness, cough and sneezing, were also significantly higher (p=0.026) in car painters. The average FVC and FEV_1 were not different between exposed and control groups.

Seventeen of 36 car painters with workplace complaints and reliable serial peak expiratory flow rate (PEFR) monitoring demonstrated a 3-week work-related pattern of positive PEFR variability of ≥20% (Eifan *et al.*, 2005). Of these, nine had current

wheezing which was considered compatible with a diagnosis of occupational asthma. Twelve of the 17 adolescents with PEFR variability ≥20% were given a methacholine inhalation test. Three of these subjects had a PC_{20} ≤8 mg/ml, which indicated a diagnosis of occupational asthma. Although smoking was identified as a possible confounder, the authors concluded that an average of 3 yrs of working duration is sufficient for developing occupational asthma in adolescent car painters.

6.3 Chronic Toxicity to Experimental Animals

6.3.1 Pathology/Histopathology Studies

HDI Monomer Chronic Toxicity Studies

In a 3-week exposure study, male and female Sprague-Dawley rats (10 rats/sex/level) were exposed head-only to monomeric HDI vapor at concentrations of 0, 0.005, 0.0175, 0.150, and 0.300 ppm (0.03, 0.12, 1.03, and 2.06 mg/m^3) for 5 hr/day, 5 days/week (Sangha, 1984). Five rats/sex/level were sacrificed and examined at the end of the 3-week exposure period, while the other 5 rats/sex/level were sacrificed following a 2-week recovery period. Daily observations noted that sneezing started during the first week of the study in rats exposed to 0.300 ppm HDI, and during the last week of exposure in rats exposed to 0.150 ppm HDI. Sneezing was attributed to severe nasal irritation. Varying degrees of eye irritation were also observed due to exposure. The observed severity of sensory irritation in rats exposed to 0.005 ppm HDI was reported to be similar to control animals. After 3-week exposure, no exposure-related effects on body weights, clinical chemistry, urinalysis, hematology, and organ weights were observed up to 0.150 ppm. At 0.300 ppm, absolute and relative kidney weights were decreased in males and females. Absolute and relative liver weights were also decreased in females at this dose level. However, the authors appeared to disregard this effect on organ weight, at least in part because the 0.300 ppm animals were exposed at a later date and compared to controls used at an earlier date.

Microscopic findings by Sangha (1984) were confined to the nasal epithelium, with some minor changes to the larynx and trachea. No exposure-related effects were found in the lungs. Five to six transverse sections of the head (i.e., nasal region) were taken at 2 to 3 mm intervals and then processed for histopathological examination. Table 11 shows the incidence and mean severity scores for the major histopathological findings identified in the upper respiratory system. The authors did not identify lesions by nasal section, but rather provided general overall findings. Consistent gender-related differences were not found, so the male and female nasal findings were combined. The authors reported that the nasal changes occurred in a dose-dependent manner with clear effects at 0.150 and 0.300 ppm in rats. The effects observed at 0.0175 ppm were

considered to be equivocal, with the NOEL established at 0.005 ppm. While the incidence of nasal changes shown in Table 11 were observed in a concentration-dependent manner, the severity of the changes did not increase with concentration for all the specified nasal effects. Changes in the larynx and trachea included focal accumulations of mixed inflammatory cells in the submucosa and a minimal to mild hyperplasia of the epithelium.

Table 11. Incidence[a] and mean severity scores[b] of the major nasal lesions in rats exposed to monomeric HDI for 3 weeks (Sangha, 1984)

Lesion Male and female results combined	HDI Concentration (ppm)				
	0	**0.005**	**0.0175**	**0.150**	**0.300**
Nasal Region					
Squamous metaplasia Incidence (severity effect mean)	0 (0.0)	1 (1.0)	5* (1.4)	10*** (2.3)	10*** (2.3)
Epithelial necrosis Incidence (severity effect mean)	3 (2.0)	3 (2.33)	7 (2.14)	10** (2.7)	10** (2.3)
Hemorrhage Incidence (severity effect mean)	3 (2.67)	2 (2.5)	2 (2.0)	4 (3.0)	6 (1.83)
Acute inflammation Incidence (severity effect mean)	0 (0.0)	1 (1.0)	1 (1.0)	7** (1.86)	9*** (2.22)
Larynx					
Laryngitis – chronic inflammation Incidence (severity score)	0 (0.0)	1 (1.0)	1 (1.0)	3 (1.67)	1 (1.0)
Trachea					
Tracheitis Incidence (severity score)	1 (1.0)	4 (1.5)	4 (1.25)	6* (1.17)	5 (2.6)
Epithelial hyperplasia Incidence (severity score)	2 (2.0)	2 (1.0)	5 (1.6)	5 (1.6)	6 (1.5)

[a] Incidence based on 10 animals per exposure level (5 males and 5 females each). Difference from control incidence calculated by OEHHA using Fisher's Exact Test (one-tailed):
* $p<0.05$; ** $p\leq0.01$; *** $p\leq0.001$

[b] Severity scoring: 0 - not remarkable, 1 – minimal, 2 – mild, 3 – moderate, 4 – marked. Severity effect mean was calculated only for animals that showed the lesion (i.e., scores 1-4)

In rats allowed a 2-week recovery period following cessation of exposure, most of the nasal epithelial lesions induced by HDI exposure had significantly regressed in females and in males in the lower exposure groups (Sangha, 1984). No recovery was apparent

in males in the high exposure group. Goblet cell hyperplasia was still observed in the 2-week recovery animals at the two highest exposure levels, mainly in male rats.

What appears to be a reexamination of the Sangha (1984) 3-week exposure study was carried out by Shiotsuka *et al*. (2006) and published in a peer-reviewed journal. Only the first four exposure levels (0, 0.005, 0.0175, and 0.150 ppm) were examined. As with the earlier study, exposure-related changes were confined to the nasal region. Greater detail was provided by Shiotsuka *et al*. regarding findings at different levels of the nasal region, although a quantified summary of the histopathological results was not presented. Squamous metaplasia, epithelial hyperplasia and goblet cell hyperplasia were significantly increased ($p<0.05$) at 0.005 ppm in the nasal epithelium of the post-incisor region. However, these lesions were significantly increased only at 0.150 ppm in the adjacent posterior region, the prepapilla region. Chronic active inflammation was increased significantly at 0.150 ppm in the vestibule, and at 0.0175 ppm in the prepapilla region. Chronic active inflammation decreased in incidence or severity in sections posterior to the prepapilla region. Ulcerative changes were noted only at the highest concentration in the anterior regions of the nasal cavity. Degeneration of the olfactory epithelium occurred at 0.150 ppm at the level of the incisor papilla, first palatial ridge and the second palatial ridge.

In animals that were sacrificed two weeks following exposure, the most notable changes were decreased incidence of ulceration, chronic active inflammation and hyperkeratosis in anterior regions, including the vestibule and post-incisor region (Shiotsuka *et al.*, 2006). Marginal recovery of squamous metaplasia was observed in the post-incisor region, with more obvious recovery in the prepapilla region. Goblet cell hyperplasia, characterized by the authors as a subtler adaptive epithelial response to injury, was increased in both male and female rats. Epithelial hyperplasia was still prominent in the prepapilla region, particularly at turbinate tips. No recovery of olfactory epithelium degeneration was observed in the incisor papilla, first palatial ridge, and second palatial ridge sections.

The persistence of epithelial cell and goblet cell hyperplasia two weeks after exposure ended was expected by the authors, since cellular proliferation is viewed to reflect a reparative or adaptive compensatory process and not necessarily a progression to an adverse effect (Shiotsuka *et al.*, 2006). Lack of recovery of olfactory epithelium suggested to the authors that HDI resulted in extensive damage to this region of the nasal epithelium. Considering all nasal lesions observed, Shiotsuka *et al*. (2006) designated degeneration of the olfactory epithelium as the critical adverse effect with a NOAEL of 0.0175 ppm. Histopathologic changes found in rats exposed to 0.005 ppm (squamous metaplasia, epithelial and goblet cell hyperplasia) were considered reversible tissue changes and not adverse in nature.

In a 13-week subchronic exposure study, male and female Fischer 344 rats (20 rats/sex/level) were exposed whole-body to monomeric HDI vapor at concentrations of 0, 0.01, 0.04 or 0.14 ppm (0.07, 0.3, or 0.96 mg/m^3), for 6 hr/day, 5 days/week (Shiotsuka, 1988). Ocular irritation, including lacrimation, was present in all rat groups but occurred in greater incidence (3- to 4-fold higher) in rats exposed to HDI. However, a dose-dependent increase in ocular irritation was not apparent. No exposure-related effects on mortality, body weight, clinical chemistry, hematology, urinalysis, gross pathology, organ weight, and organ-to-body weight ratios were observed. Histopathological examination found exposure-related nasal lesions, including hyperplasia and/or squamous metaplasia of the respiratory epithelium, mucous cell hyperplasia mainly in respiratory epithelium, and inflammatory cell infiltrate mainly in subepithelial tissues (Table 12). Keratin covering metaplastic epithelium was observed at the two highest concentrations. Lesions were primarily found in the anterior nasal cavity region at all exposure levels, although the lesions at 0.01 ppm were considered minor and present in only a few animals. Thus, the pathologist considered 0.01 ppm to be a near-threshold level. The mean severity of both hyperplasia and squamous metaplasia generally increased with dose and were considered the most important exposure-related findings of the study. Only minimal degenerative changes in olfactory epithelium were seen in two males exposed to 0.14 ppm HDI, and no compound-related degenerative changes were observed in the larynx, trachea, or lungs.

Table 12. Incidence[a] and mean severity scores of major nasal lesions in rats exposed to monomeric HDI for 13 weeks (Shiotsuka, 1988)

Nasal Lesion (Level)[b]	HDI Concentration (ppm)			
	0	0.01	0.04	0.14
Hyperplasia – males (III) Incidence (Severity effect mean)[c]	2 (1.0)	1 (1.0)	15*** (1.53)	19*** (3.0)
Hyperplasia – females (III) Incidence (Severity effect mean)	0 (0.0)	5* (2.0)	16*** (2.13)	20*** (3.2)
Squamous metaplasia – males (III) Incidence (Severity effect mean)	0 (0.0)	2 (1.0)	2 (1.5)	16*** (1.81)
Squamous metaplasia – females (III) Incidence (Severity effect mean)	0 (0.0)	4 (1.0)	7** (2.0)	17*** (2.0)
Mucous hyperplasia – males (III) Incidence (Severity effect mean)	0 (0.0)	1 (1.0)	2 (1.0)	2 (1.5)
Mucous hyperplasia – females (III) Incidence (Severity effect mean)	0 (0.0)	1 ([d])	4 ([d])	3 ([d])
Inflammation – males (II) Incidence (Severity effect mean)	9 (1.67)	5 (1.6)	8 (1.13)	12 (1.08)
Inflammation – females (II) Incidence (Severity effect mean)	9 (1.67)	10 (1.3)	14 (1.71)	20*** (1.80)

[a] Incidence based on 20 animals/sex/exposure level. Difference from control incidence calculated by OEHHA using Fisher's Exact Test (one-tailed) * $p<0.05$; ** $p\leq0.01$; *** $p\leq0.001$
[b] The level refers to the nasal level of sectioning. Level II - posterior to incisor; Level III - midpoint between incisive papilla and incisor teeth and posterior
[c] Severity effect mean calculated only for animals that demonstrated the lesion (See Table 11 for severity scoring scheme)
[d] Could not be determined due to poor manuscript reproduction

In a chronic exposure study, male and female Fischer 344 rats (60 rats/sex/level) were exposed whole-body to monomeric HDI vapor at concentrations of 0, 0.005, 0.025, and 0.164 ppm (0.03, 0.17, and 1.13 mg/m^3) for 6 hr/day, 5 days/week for 2 yrs (Shiotsuka, 1989; Shiotsuka *et al.*, 2010). A satellite group of rats (10 rats/sex/level) was exposed under the same conditions for one year. No compound-related effects on mortality occurred during the study. Exposure-related clinical signs were observed only at 0.164 ppm and consisted of irritated eyes in male rats (i.e., primarily increased lacrimation) during the first year of exposure. However, no chemical-related eye lesions were detected in either male or female rats upon ophthalmoscopic examination. Female rats

in the high exposure group had a consistently lower average body weight (p<0.05) of about 5% compared to the control group during the second year of exposure. Increased reticulocytes in high exposure male and female groups were observed at several intervals, suggestive of anemia, and were considered a borderline effect. Additionally, females in the high exposure group at 24 months exhibited decreased total RBC, hematocrit, hemoglobin, and mean corpuscular hemoglobin concentration values with significantly increased reticulocytes and mean corpuscular volume. No exposure-related differences in clinical chemistry and urinalysis indices were observed. Gross pathology and organ weights did not reveal any differences between exposure groups.

The principal target organ for both the 1-year satellite and 2-year exposure groups was the nasal cavity (Shiotsuka, 1989; Shiotsuka *et al.*, 2010). Although beyond the scope of this document, no evidence of carcinogenic activity was found in the respiratory tract, or in other organs, of HDI-exposed rodents. Major histopathologic findings included hyperkeratosis, epithelial hyperplasia, mild to moderate diffuse squamous metaplasia, chronic active inflammation, mucus secretory cell hyperplasia, hyaline droplet degeneration, and degeneration of the olfactory epithelium. Overall, an anterior to posterior gradient of incidence and/or severity of these nasal findings was observed. The nasal lesions of particular importance to the authors were chronic active inflammation and olfactory epithelium degeneration (Table 13). Chronic active inflammation showed a marked anterior to posterior gradient of incidence from the 1-year to 2-years of exposure. Olfactory epithelium degeneration showed a progression of increased incidence and severity within the same anatomic region from 1- to 2-years of exposure. A dose-dependent, statistically significant increased incidence of these lesions was apparent starting at 0.025 ppm. Only the female rat incidence and severity scores for chronic active inflammation are shown in Table 13. Female rats tended to have a greater background level and greater HDI sensitivity for this lesion compared to male rats. For olfactory epithelial degeneration, male rats tended to be more sensitive than female rats with exposure to HDI.

Table 13. Incidence[a] and mean severity scores[b] of major nasal lesions in rats exposed to monomeric HDI for 2 years (Shiotsuka, 1989)

Nasal Lesion (Level)[c]	HDI Concentration (ppm)			
	0	0.005	0.025	0.164
Olfactory epithelium degeneration (IV)				
Number examined – males	60	60	60	59
Incidence (Severity effect mean)	0 (0.0)	0 (0.0)	7**(1.14)	50***(1.90)
Olfactory epithelium degeneration (V)				
Number examined – males	59	60	60	59
Incidence (Severity effect mean)	0 (0.0)	2 (1.50)	8**(1.50)	54***(2.26)
Chronic active inflammation (I)				
Number examined – females	59	60	60	60
Incidence (Severity effect mean)	40 (1.20)	49 (1.29)	42 (1.24)	41 (1.17)
Chronic active inflammation (II)				
Number examined - females	59	60	60	60
Incidence (Severity effect mean)	38 (1.55)	46 (1.39)	55***(1.56)	59***(2.12)
Chronic active inflammation (III)				
Number examined – females	58	60	60	60
Incidence (Severity effect mean)	18 (1.17)	25 (1.12)	30* (1.07)	40***(1.13)
Chronic active inflammation (IV)				
Number examined - females	58	60	60	60
Incidence (Severity effect mean)	22 (1.23)	16 (1.06)	38**(1.08)	34* (1.09)
Chronic active inflammation (V)				
Number examined – females	58	59	60	59
Incidence (Severity effect mean)	18 (1.33)	16 (1.06)	29* (1.21)	38***(1.34)

[a] Difference from control incidence calculated by OEHHA using Fisher's Exact Test (one-tailed) * $p<0.05$; ** $p\leq0.01$; *** $p\leq0.001$

[b] Histopathology was scored using an ordinal scale from Grade 0 (normal) to Grade 5 (severe). Severity effect mean calculated only for animals that showed the lesion (severity scores 1 to 5)

[c] The level refers to the nasal level of sectioning. Level 1 = anterior incisor; Level 2 = posterior incisor; Level 3 = midpoint between incisors and incisive papilla; Level 4 = incisive papilla; Level 5 = first palatal ridge

Table 14 presents the incidence and mean severity score for what was described as the adaptive lesions resulting from 2 year exposure to HDI (Shiotsuka, 1989). Five or six transverse sections of the nasal region were examined. Exposure-related changes to nasal tissue were mostly observed at level II (posterior incisor) and level III (midpoint between incisors and incisive papilla). The incidence and severity of squamous

metaplasia were increased only at 0.164 ppm in Level II males and females, and at Level V in males (data not shown). Hyperkeratosis was also observed primarily in the high exposure groups (data not shown). The incidence of epithelial hyperplasia/metaplasia was increased in the 0.005 and 0.025 ppm groups at Level I (data not shown) and Level II (Table 14), but was at or below control levels at the highest exposure. A similar concentration-response relationship was observed for incidence of hyaline droplet formation in male and females. However, the mean severity score for epithelial hyperplasia/metaplasia increased with increasing dose, while the mean severity score for hyaline droplet formation increased in the two lowest exposure groups, but decreased in the high exposure group. Shiotsuka (1989) notes that the incidence of hyaline droplet formation was also significantly increased at 0.005 ppm in Level IV sections (data not shown). The incidence of mucus hyperplasia was significantly increased in the 0.025 and 0.164 ppm male and female exposure groups at Level II and III, and in the 0.005 ppm females at Level III.

The nasal lesions presented in Table 14 were not considered adverse by the authors or were not a sensitive indicator of injury (Shiotsuka *et al*., 2010). Because these lesions were viewed as adaptive responses and dose-dependency was often not seen, Shiotsuka (1989) concluded that 0.005 ppm appeared to be a subtle exposure effect level (i.e., a NOAEL, but no NOEL in this study) that was evidence of a protective response to non-specific irritation.

Table 14. Incidence[a] and mean severity scores[b] of squamous metaplasia, epithelial hyperplasia/metaplasia, hyaline droplet formation and mucus hyperplasia in rats exposed to monomeric HDI for 2 years (Shiotsuka, 1989)[a]

Pulmonary Lesion (Level)[c]	HDI Concentration (ppm)			
	0	0.005	0.025	0.164
Squamous metaplasia (II)				
Number examined – sexes combined	119	120	120	119
Incidence (Severity effect mean)	13 (2.23)	14 (2.14)	10 (2.30)	97***(3.13)
Epithelial hyperplasia/metaplasia (II)				
Number examined - males	60	60	60	59
Incidence (Severity effect mean)	14 (1.50)	30**(1.23)	53***(1.79)	2**(2.50)
Epithelial hyperplasia/metaplasia (II)				
Number examined - females	60	60	60	60
Incidence (Severity effect mean)	10 (1.80)	35***(1.57)	55***(2.49)	20 (3.05)
Hyaline droplet formation (III)				
Number examined - males	60	60	60	59
Incidence (Severity effect mean)	2 (1.00)	7 (1.29)	31***(1.61)	1 (1.00)
Hyaline droplet formation (III)				
Number examined - females	59	60	60	60
Incidence (Severity effect mean)	13 (1.08)	41***(1.51)	54***(1.69)	16 (1.29)
Mucus hyperplasia (III)				
Number examined - males	60	60	60	59
Incidence (Severity effect mean)	8 (1.50)	16 (1.19)	28***(1.36)	37***(1.46)
Mucus hyperplasia (III)				
Number examined - females	59	60	60	60
Incidence (Severity effect mean)	9 (1.11)	20* (1.20)	37***(1.35)	30***(1.30)

[a] Difference from control incidence calculated by OEHHA using Fisher's Exact Test (two-tailed) * $p<0.05$; ** $p\leq0.01$; *** $p\leq0.001$

[b] Severity effect mean calculated only for animals that showed the lesion (severity scores 1 to 5)

[c] The level refers to the nasal level of sectioning. Level 2 = posterior incisor; Level 3 = midpoint between incisors and incisive papilla

Shiotsuka (1989) did not provide an explanation or theory for the decreased incidence epithelial hyperplasia/metaplasia and hyaline droplet formation at the high HDI concentration. OEHHA speculates that the increased injury to the epithelium at the high HDI concentration results in a change in cellular response. This cellular response may decrease the incidence of epithelial hyperplasia/metaplasia and hyaline droplet

formation in favor of other effects, such as squamous metaplasia and mucus hyperplasia.

Some pulmonary effects were noted by Shiotsuka (1989), although the incidences and severity scores determined by OEHHA from the original data were considerably lower (Table 15) than many of the nasal lesion findings. Shiotsuka (1989) reported slightly increased incidences of lung lesions (alveolar lining cell proliferation, interstitial pneumonia, and alveolar macrophage accumulation) in both males and females in the 0.025 and 0.164 ppm exposure groups. Examination of the original data by Shiotsuka (1989) suggests that little or no alveolar epithelization resulted from HDI exposure. Interstitial pneumonia was increased at the two highest exposure levels, and alveolar macrophage accumulation occurred only at the highest exposure. No associated exposure-related lesions were found in the trachea, larynx, or nasal lacrimal duct.

Table 15. Incidence[a] and mean severity scores[b] of pulmonary lesions in rats exposed to monomeric HDI for 2 years (Shiotsuka, 1989)

Pulmonary Lesion Male and female results combined	HDI Concentration (ppm)			
	0	0.005	0.025	0.164
Alveolar epithelialization				
Number examined	118	120	120	120
Incidence (Severity effect mean)	2 (2.50)	1 (3.00)	4 (1.75)	2 (1.00)
Interstitial pneumonia				
Number examined	118	120	120	120
Incidence (Severity effect mean)	11 (1.36)	11 (2.00)	22* (2.18)	25**(1.60)
Alveolar macrophages				
Number examined	118	120	120	120
Incidence (Severity effect mean)	20 (1.05)	27 (1.00)	24 (1.08)	34* (1.09)

[a] Difference from control incidence calculated by OEHHA using Fisher's Exact Test (one-tailed) * $p<0.05$; ** $p\leq0.01$

[b] Severity effect mean calculated only for animals that showed the lesion (severity scores 1 to 5)

HDI Polyisocyanate Chronic Toxicity Studies

In a three week range finding study, groups of male and female Wistar rats were exposed to either HDI isocyanurate or biuret (10/sex/group/compound) at 3, 15, and 75 mg/m^3 for 6 hr/day, 5 days/week to examine the pulmonary and extrapulmonary effects (Pauluhn and Mohr, 2001). The MMAD ± GSD ranges of the prepolymer aerosols generated were 1.4-2.5 µm ± 1.3-2.1. At study termination, lung wet weights were significantly increased ($p<0.01$) at the two highest exposure levels. Other major organ weights were unchanged. Urinalysis and hematological and clinical chemical

determinations were unremarkable. Adverse effects were confined to the respiratory system. Gross examination of rats exposed to 75 mg/m^3, and to a lesser extent at 15 mg/m^3, revealed dark red, distended lungs with occasional foamy mucus in their tracheas. At 75 mg/m^3, histopathological examination of rats exposed to either prepolymer revealed focal hyperplasia in the larynx and trachea, and inflammation, fibrosis, thickening of septa and increased influx of alveolar macrophages in the bronchioloalveolar (i.e., bronchoalveolar) region. Nasal tissue changes at the high concentration consisted of goblet cell hypertrophy and hyperplasia. Similar respiratory tract changes at the intermediate level were found to a lesser extent in rats exposed to biuret, but appeared to be absent at this concentration in rats exposed to isocyanurate. No irritant-related respiratory tract changes were observed at 3 mg/m^3.

In the follow-up study, groups of male and female Wistar rats were exposed to either HDI isocyanurate or biuret (10/sex/group/compound) nose-only at concentrations of 0.4, 3, and 25 mg/m^3 for 6 hr/day, 5 days/week for 13 weeks (Pauluhn and Mohr, 2001). The MMAD ± GSD ranges for biuret aerosol were 1.4-3.3 µm ± 1.3-1.6 (Table 16). For isocyanurate, the MMAD ± GSD ranges were 1.4-1.5 µm ± 1.3-1.6. Lung wet weights were increased (p<0.01) at the high exposure for rats exposed to isocyanurate. Consistent with larger particle size at the high dose (MMAD = 3.3 µm), minimal lung wet weight increases were seen in rats exposed to biuret (p<0.05, males only).

Table 16. MMAD and GSD of chamber HDI prepolymer aerosols of biuret and isocyanurate for 13-week exposure study in rats (Pauluhn and Mohr, 2001)

HDI Prepolymer	Chamber Concentration (mg/m^3)		
	0.4	3	25
Biuret			
MMAD	1.4 ± 0.2	1.5 ± 0.2	3.3 ± 0.2
GSD	1.3 ± 0.1	1.4 ± 0.0	1.6 ± 0.2
Isocyanurate			
MMAD	1.5 ± 0.0	1.4 ± 0.0	1.5 ± 0.1
GSD	1.4 ± 0.0	1.3 ± 0.0	1.6 ± 0.1

Urinalysis, hematological, and clinical chemical determinations were unremarkable, except for a slight increase in relative leukocyte counts at high exposures, but without associated changes in differential blood counts. Lung function measurements, including functional residual capacity, total lung capacity and acetylcholine bronchoprovocation, could not detect any changes among the exposure groups. Pathological changes were confined to the respiratory tract. Histopathologic examination revealed bronchioloalveolar lesions only at 25 mg/m^3, including increased number of alveolar macrophages, thickening of septa, fibrosis and bronchioloalveolar proliferation (Table

17). No adverse effects were observed in nasal tissue. Overall, the toxicological responses in males and females were similar for both prepolymers.

Table 17. Incidence of pulmonary lesions in rats exposed to HDI biuret and isocyanurate prepolymers for 13 weeks (Pauluhn and Mohr, 2001)

Pulmonary Lesion[a]	Prepolymer Concentration (mg/m^3)			
	0	0.4	3	25
HDI Biuret				
Increased number of alveolar macrophages	1	1	0	6*
Thickening of septa	0	0	1	7**
Fibrosis	0	0	1	4
Bronchioloalveolar proliferation	0	0	0	16**
HDI Isocyanurate				
Increased number of alveolar macrophages	0	0	1	20**
Thickening of septa	0	0	1	8**
Fibrosis	0	0	1	8**
Bronchioloalveolar proliferation	1	0	1	20**

[a] Incidence based on 20 animals per exposure level.
* $p<0.05$; ** $p<0.01$ for difference from control incidence by Fisher's Exact Test (two-tailed)

Combined with earlier acute studies with HDI isocyanurate, Pauluhn and Mohr (2001) concluded that the NOAELs for both isocyanurate and biuret are in the range of 3-4 mg/m^3, whether the exposure of rats was acute, subacute, or subchronic. Overall the authors concluded that both HDI prepolymers had almost equivalent toxic potencies, and that any differences in toxic potency were considered related to differences in particle size.

6.3.2 Respiratory Sensitization Studies

To study the asthmogenic potential of HDI monomer and polyisocyanates, guinea pigs have been used in studies due to their propensity to develop a strong bronchoconstrictive response upon challenge with allergens. A recent report by Pauluhn (2015) also supports Brown Norway rats as a good animal model for respiratory sensitization studies.

Airway hyperresponsiveness was assessed in a control group and two groups of Hartley-Dunkin guinea pigs that were exposed to 0.01 ppm (0.069 mg/m^3) monomeric HDI for 6 hr/day, 5 days/week for 8 weeks (Marek *et al.*, 1997). This level of HDI exposure did not significantly alter basal values of respiratory mechanical and cardiovascular parameters. After the last HDI exposure, nonspecific airway responsiveness was assessed with acetylcholine (ACh) in one exposed group of guinea pigs. Increased airway constriction compared to the control group ($p<0.005$), measured

by changes in dynamic elastance, occurred at 1.0 and 2.0% ACh, but not less. Challenge to a concentration of 0.01 ppm HDI for 60 min did not cause any significant changes in functional parameters. The second group of HDI-exposed guinea pigs was challenged with ACh after an 8-week latency period. Airway hyperresponsiveness to ACh challenge was similar to control animals and significantly smaller (p<0.005) than animals that were challenged immediately after HDI exposure. The authors concluded that the lack of response to HDI challenge may be the result of the low HDI concentration and short exposure duration. They also concluded the transient airway hyperresponsiveness to ACh suggests an initial, but reversible, step in the development of isocyanate-induced asthma with 8 weeks of exposure to HDI.

Pauluhn *et al*. (2002) compared the sensitization potential of HDI monomer with the sensitization potential of the HDI biuret and isocyanurate prepolymers. Guinea pigs were sensitized by inhalation of 27 mg/m^3 (4 ppm) HDI vapor 3 hr/day for 5 consecutive days, or by repeated intradermal injections (3x, 0.3% HDI) on days 0, 2 and 4. For the prepolymers, groups of guinea pigs were sensitized by inhalation of the aerosols (biuret: 0, 3, 10, 48, and 142 mg/m^3; isocyanurate: 0, 3, 16, 49, and 261 mg/m^3) using the same protocol. Prepolymer MMAD and GSD were in the range of 1.5-2.0 and 1.4-1.5 µm, respectively. For guinea pigs sensitized by intradermal injections, prepolymer exposures were greater (6 injections of 30% solutions) than that used for the monomer (3 injections of a 0.3% solution). Guinea pigs sensitized and challenged with HDI 2-3 weeks later did not show an increased respiratory rate whereas challenge with HDI-guinea pig serum albumin conjugate caused a marked increase in respiratory rate. Both the intensity of response and the incidence of responding animals were highest in intradermally sensitized animals. The authors presumed that the negative outcome for the HDI challenge was due to the low challenge dose of 0.5 mg/m^3 (0.07 ppm) used to avoid irritant-related changes in respiratory rate. This resulted in HDI vapor being scrubbed in upper airways, thus reducing the effective bronchial dose.

Guinea pigs sensitized with either prepolymer did not show marked changes in respiratory responses when challenged with the haptens or the conjugate of the haptens (Pauluhn *et al.*, 2002). In lung tissue sections, bronchial inflammation characterized by infiltration of eosinophilic granulocytes was only seen in guinea pigs sensitized to HDI and challenged with free and conjugated HDI. Assessment of IgG_1-antibody titer was also conducted. Monomeric HDI was found to be more potent in inducing specific IgG_1 antibodies than the HDI prepolymers. The authors concluded that the asthmogenic potency of the prepolymers was insignificant compared to that of the monomer.

Pauluhn (2015) developed a respiratory sensitization/elicitation protocol in Brown Norway rats to determine a threshold dose of HDI for elicitation of asthma-like

responses in sensitized, re-challenged rats. Unlike TDI and MDI, the physicochemical properties of HDI vapor favor its retention predominantly in the upper airways (Pauluhn, 2008a; Schroeter *et al.*, 2013; Pauluhn, 2014; 2015). Thus, an inhaled dose above the upper airway irritant threshold must be used in order for HDI to reach susceptible sites (i.e., bronchial airways) for elicitation and sensitization. Several equally spaced priming/aggravation inhalation exposures to mildly alveolar irritant concentrations of HDI were used at exposure durations long enough to deliver a sufficiently high inhaled dose to distal airways of the lung (Pauluhn, 2015). The resulting doses needed for HDI to gain access to lower airways occurred at concentrations in which there is a shift in the vapor-to-aerosol equilibrium towards the aerosol phase, approximately 120 mg/m^3.

Previous work found that skin-sensitization with MDI or TDI produced a more pronounced subsequent response than repeated inhalation-only sensitization upon inhalation challenge with the respective diisocyanate (Pauluhn and Poole, 2011; Pauluhn, 2014), so a similar protocol was used for HDI. For the respiratory sensitization/elicitation protocol, groups of rats dermally sensitized with 2%-HDI on days 0 and 7 were exposed to a dose-escalation type ($C \times t$) of bronchoprovocation at 110 mg/m^3 HDI for either 6, 13, 35 or 50 min on day 20 (Pauluhn, 2015). In addition, groups of similarly sensitized rats were exposed to three successive inhalation priming/challenge exposures at about 120-87-72 mg/m^3 × 30 min followed by bronchoprovocation challenge at about 72 mg/m^3 for either 6, 13, 35, or 75 min. Lung priming challenges occurred on days 20, 35 and 50, with bronchoprovocation challenge occurring on day 65.

Neutrophilic granulocytes (PMN) in BAL fluid were used as the endpoint for allergic pulmonary inflammation in the rats. This endpoint is associated with asthma-like pathophysiological responses delayed in onset in both humans and rats. This was supplemented by measurement of nitric oxide in exhaled breath, determined shortly after each final provocation challenge and 20 hours later. A dose-dependent increase in BAL-PMN was observed particularly when using the repeated inhalation priming protocol. Rats in the naïve control group not receiving repeated priming inhalation exposures did not show any escalation dose-dependent increase in BAL-PMN whereas those naïve control rats repeatedly primed showed increased susceptibility. Nitric oxide in exhaled breath did not reveal significant differences between sensitized and non-sensitized rats. A benchmark dose approach was used to determine the NOAEL for elicitation. Based on the repeated priming/challenge protocol, which provided a better benchmark dose fit and used less irritant challenge doses, BAL-PMN in equally challenged naïve and HDI-sensitized rats were considered indistinguishable at 900 mg HDI/m^3 × min (129 ppm × min).

7. Developmental and Reproductive Toxicity

Groups of 25 pregnant Sprague-Dawley rats were chamber-exposed to HDI monomer at concentrations of 0, 0.005, 0.05, and 0.300 ppm (0.034, 0.34 and 2.1 mg/m^3) continuously on days 0-19 of gestation (Astroff *et al.*, 2000a). Dose-dependent maternal toxicity was limited to the nasal turbinates of rats exposed to 0.05 and 0.300 ppm HDI (p<0.05). Specific effects included acanthosis (i.e., thickening of the squamous epithelial layer), hyperkeratosis, inflammation of the nasal turbinates, and degeneration of the olfactory epithelium. Maternal histopathological and/or gross lesions were scored using a semiquantitative ranking of the severity grade/stage (Table 18). Multiple sections of the nasal turbinates were preserved and examined. In the most anterior region of the nose, the vestibule, minimal (grade 1) acanthosis and inflammation was observed at 0.005 ppm in only a few animals. No other histopathological findings were observed at this concentration in more posterior sections of the nose. No micropathological findings were observed in the nasopharynx section of the turbinates, or in the larynx, trachea, and lung. The authors concluded that no maternal toxicity was observed at the lowest concentration of 0.005 ppm.

Table 18. Incidence and grade of nasal lesions in the vestibule of pregnant female rats exposed continuously to HDI monomer on days 0-19 of gestation (Astroff et al., 2000a), and 6 hrs/day for up to 50 days during pre-mating, mating and gestational days (Astroff et al., 2000b)

Pulmonary Lesion[a]	HDI Concentration (ppm)			
	0	0.005	0.050	0.300
Astroff et al., 2000a				
Number examined	20	23	25	25
Vestibule section				
Acanthosis	0/0[a]	2/1.0	13/1.0*	15/1.2*
Hyperkeratosis	0/0	0/0	3/1.3	25/1.2*
Inflammation (chronic-active)	0/0	1/1.0	5/1.0*	24/1.2*
Astroff et al., 2000b				
Number examined	15	15	15	14
Vestibule section				
Acanthosis	1/1.0	1/1.0	1/1.0	5/1.0
Hyperkeratosis	0/0	0/0	1/1.0	2/1.0
Inflammation (chronic-active)	0/0	0/0	0/0	4/1.0*
Erosion	0/0	0/0	0/0	2/1.0

[a] Number of animals with lesion/median severity grade 0 – normal; grade 1 – minimal; grade 2 - mild or slight; grade 3 – moderate; grade 4 – marked; grade 5 - severe.

* Incidence significantly greater than control ($p < 0.05$).

Reproductive, implantation and fetal weight endpoints were unaffected by exposure (Astroff *et al.*, 2000a). No exposure-related effects on fetal and litter incidence of external and visceral malformations or variations were observed. A statistically significant increase in the fetal incidence, but not the litter incidence, of incomplete ossification of the frontal bone was noted at 0.300 ppm. No other skeletal findings were observed. The authors concluded there was no evidence of developmental toxicity in the study.

A combined reproduction, developmental and neurotoxicity study was also conducted in Sprague-Dawley rats at HDI concentrations of 0, 0.005, 0.05, and 0.300 ppm (0.034, 0.34 and 2.1 mg/m^3) (Astroff *et al.*, 2000b). Exposures were for 6 hr/day during a 14-day premating phase, during the mating phase that was up to 14 days, and during a 21-day gestation phase at which time HDI exposure was discontinued. Final measurements were taken at termination on Lactation Day 4. Mean body weights in males and females exposed to 0.300 ppm during the premating phase were reduced only on Day 4. Body weights at other times, and food consumption and clinical signs were unaffected by HDI exposure during premating and mating phases. No effects were observed for mating, fertility, or gestation indices. No effect was observed on litter indices including litter size, number of litters, viability, gender distribution, and live births. Pup growth, as measured by body weight and body weight gain, did not differ among the exposure groups during the final four days of lactation.

Adult male and female rats from each dose level underwent a repeated functional observational battery and motor activity testing in the final days of the premating phase (Astroff *et al.*, 2000b). A final evaluation of female rats was conducted before termination on Lactation Day 4. Landing foot splay, grip strength, and motor and locomotor activity measured in a figure 8 maze were among the tests administered. No compound-related differences in neurobehavioral parameters were found. Hematology and clinical chemistry endpoints were also unaffected by exposure. All major organs, including reproductive organs, were examined. Multiple sections of the nasal turbinates were preserved and examined. Organ effects were limited to microscopic lesions of the upper respiratory tract, mainly in nasal turbinates, at the two highest concentrations (Table 18). The histopathologic findings were more severe in females than in males, which the authors suggested was due in part to the increased respiratory rate typically observed during gestation. The authors concluded that the NOAEL for HDI exposure in adult animals was 0.005 ppm, and that HDI at the concentrations tested did not elicit any effects on reproduction, gestation, or early neonatal development.

8. Toxicogenetics

It has been shown that 5 to 15% of exposed workers may develop isocyanate-induced asthma. Thus, genetic variability has been implicated in the susceptibility to occupational asthma by HDI polyisocyanates and other isocyanates. A number of gene variants have been reported to be associated with increased sensitivity to the disease in workers (Table 19), which suggests that isocyanate-induced asthma represents a complex disease phenotype determined by multiple genes. Examples include, but are not limited to, genes involved in immune regulation, inflammatory regulation, and antioxidant defense (Choi *et al.*, 2009; Yucesoy and Johnson, 2011; Yucesoy *et al.*, 2012). The goal of genetic association studies is to provide more accurate information on interindividual variability, thereby contributing to better protection of sensitive human populations and to the establishment of more accurate exposure limits. Since exposure to the other major commercial isocyanates (MDI and TDI) results in sensitization and asthma in susceptible individuals similar to HDI-based polyisocyanates, the combined toxicogenomic findings for all isocyanates are presented here for a more complete picture of the influence of genotype on the respiratory disease outcome resulting from isocyanate exposure.

Piirila *et al*. (2001) evaluated polymorphisms in glutathione-S-transferase genes (GSTM1, GSTM3, GSTT1, and GSTP1) to look for associations with diisocyanate-induced asthma (DA) in workers exposed to TDI, MDI, and/or HDI in a variety of occupations. The term “DA” also includes painters exposed to HDI-based polyisocyanates. There were 109 cases of workers with DA and 73 exposed non-symptomatic controls. Most (>93%) of the DA cases were diagnosed based on specific inhalation challenge tests, while the remainder were diagnosed based on lung function evaluation. Peripheral lymphocytes served as the source of DNA for genotyping. Lack of the GSTM1 gene (null) was found to be associated with increased risk of DA by regression analysis comparing workers lacking the gene to those with the gene, after controlling for age, sex, smoking, and atopy. No other GST polymorphisms in this study were related to the risk of DA. In a later study on the same worker group, Wikman *et al*. (2002) investigated the possible role of N-acetyltransferase (NAT) genotypes in the development of DA. Regression analysis revealed positive associations for increased DA with slow acetylator genotypes, especially in TDI exposed, and genotype combinations with a glutathione-S-transferase (GSTM1 null) genotype, after adjusting for age, smoking, sex, and atopy.

A case-control study was conducted by Yucesoy *et al*. (2012) to investigate whether genetic variants of antioxidant defense genes are associated with increased susceptibility to DA. The study population consisted of 353 diisocyanate-exposed (TDI,

MDI, and HDI) Caucasian French-Canadian workers recruited from occupational clinics in Canada or, in the case of asymptomatic workers, from painters in Quebec, Canada exposed to HDI. The workers were divided into three groups: 95 workers with specific inhalation challenge confirmed DA (DA+); 116 symptomatic diisocyanate workers with a negative specific inhalation challenge (DA-); and 142 asymptomatic exposed workers (AW). Specific inhalation challenge with the work-related diisocyanate resulting in a 20% drop in FEV_1 was considered positive for DA. The investigators analyzed the role of gene variants for antioxidant defense genes previously shown to modulate susceptibility to asthma and other inflammatory respiratory diseases. The investigators included epoxide hydrolase, which detoxifies epoxides, because of evidence that the EPHX genotype modulates risk of asthma, emphysema, and chronic obstructive pulmonary disease. Genotyping of peripheral blood samples allowed examination of single nucleotide polymorphisms (SNPs) in several genes, and deletion polymorphisms in GSTT1 and GSTM1.

Antioxidant defense gene variations for superoxide dismutase, glutathione-S-transferase and epoxide hydrolase and their interactions were found to contribute to DA susceptibility (Yucesoy *et al.*, 2012). Results of regression models examining statistically significant SNPs, after adjusting for age, smoking status, and duration of exposure, are presented in Table 19 for those SNPs and interactions that increased susceptibility to diisocyanate-induced asthma. Comparisons were made for gene variants that differed between the DA+ group and the DA- group as well as the DA+ group and the AW group. Odds ratios up to 10-fold are noted for the gene variants that resulted in increased sensitivity to DA (Table 19). The investigators also reported a number of gene variants that conferred protection against DA, for example, GSTM1 null and the EPHX1 rs2854450 SNP. Combinations of SNPs conferred protection or increased sensitivity, depending on the SNPs carried. These data support the hypothesis that genetic variability within antioxidant defense systems contributes to the pathogenesis of diisocyanate-induced asthma, and indicate a wide variability in susceptibility to diisocyanate-induced asthma based on genotype, including modification of susceptibility by gene-gene interactions.

Blindow *et al*. (2015) performed a pilot analysis on 27 isocyanate-exposed subjects that underwent specific inhalation challenge (SIC) tests to compare mutation frequencies in GSTM1, GSTT1 and GSTP1 genes among the subjects sorted by SIC response and total IgE levels. SIC challenge was to the isocyanate which the subjects were exposed to at work (MDI, HDI, TDI, IPDI, NDI and HDI-containing hardening agents). Although patient numbers were too low to calculate statistically significant differences in most cases, some differences were observed. Seventy-three percent of patients with more than 20 IU/ml total IgE in their blood serum (11 of 15) were GSTM1 null mutant, in

contrast with only 33% of patients with GSTM1 deletions and less than 20 IU/ml total IgE (4 of 12) (p=0.01). Mutations in GSTP1*A114V also seem to increase the risk for developing IgE-mediated reactions (4 of 15 with >20 IU/ml total IgE vs. 0 of 12 with <20 IU/ml total IgE). Also, a deletion of GSTT1 was found more often (p=0.08) in the DA+ patients (6 of 12) compared to the DA- patients (2 of 15).

The human leucocyte antigen (HLA) class II molecules are also thought to be involved in the development of the immune response to diisocyanates. HLA class II molecules are encoded by genes located within the major histocompatibility complex and present antigens from outside of the cell to T-lymphocytes. These particular antigens stimulate the multiplication of T-helper cells, which in turn stimulate antibody-producing B-cells to produce antibodies to that specific antigen. Bignon *et al*. (1994) first looked for potential associations for susceptibility or resistance to isocyanate-induced asthma with HLA Class II genes. The distribution of alleles (DQA1, DQB1, DPB1 and DRB) of the different HLA Class II genes was examined in patients of European-born workers with TDI-induced asthma (n=28) and compared to similarly exposed workers without the disease (n=16). Allele DQB1*0503 (p<0.04, RR = 9.85) and allelic combination DQB1*0201/0301 (p<0.054, RR = 9.53) were found to be significantly greater in DA group. Alternatively, allele DQB1*0501 (p<0.03, RR = 0.14) and the DQA1*0101-DQB1*0501-DR1 haplotype (p<0.016, RR = 0.056) was significantly greater in the healthy control group. These data suggest that genotype for HLA class II molecules influences risk of TDI-induced asthma.

Balboni *et al*. (1996) evaluated HLA Class II genetic markers (DQA and DQB) in 30 TDI-induced asthma cases, 12 asymptomatic exposed workers and 126 unexposed controls. A positive association was found with HLA-DQB1*0503 and a negative association found with HLA-DQB1*0501 alleles. The only difference was at residue 57 for a single amino acid, with aspartic acid in DQB1*0503 and valine in DQB1*0501. No significant difference was found in the distribution of DQA1 alleles between asthmatics and controls.

Mapp *et al*. (2000) also examined the distribution of markers (DQA, DQB and DRB) for HLA class II genes in European Caucasians (67 TDI-exposed workers with DA, 27 asymptomatic TDI-exposed worker controls, and 101 normals), and compared the results to previously generated data on 101 non-asthmatics from Northern Italy (normal subjects). The frequencies of DQA1*0104 and DQB1*0503 were significantly increased in asthmatic subjects, while DQA*0101 and DQB*0501 were significantly higher in asymptomatic exposed workers. DQB1*0503 was also more frequent among asthmatic subjects compared with normal subjects.

Kim *et al*. (2006) evaluated a Korean population for associations of HLA class I and II alleles with TDI-induced asthma (measured using TDI bronchoprovocation challenge). These investigators compared the HLA genotype, determined by direct DNA sequencing of genomic material from peripheral blood mononuclear cells, of workers with isocyanate-induced asthma (n=55), exposed asymptomatic workers (n=47) and unexposed healthy subjects (n=95). Single allele analysis did not reveal any statistically significant differences. However, two and three locus haplotype analysis showed several significant alleles as potential susceptibility markers for TDI-induced asthma. The authors identified the HLA haplotype DRB1*15-DPB1*05 as the most useful marker for predicting development of TDI-induced occupational asthma in the Korean population.

A more recent study by the same Korean research group expanded on the earlier study by looking for associations of HLA class I and II alleles with TDI-induced asthma using high resolution analysis (Choi *et al.*, 2009). The Korean study population included 84 workers with DA, 47 asymptomatic controls and 127 unexposed normal controls. DNA from peripheral blood mononuclear cells was first amplified using PCR and then subjected to DNA sequencing. No significant association was found between allele frequencies and TDI-induced asthma. However, two- and three-locus haplotype frequencies were found that were associated with TDI-induced asthma compared to both asymptomatic workers and unexposed controls (DRB1*1501-DQB1*0602-DPB1*0501, DRB1*1501- DQB1*0602, and DRB1*1501- DPB1*0501). The authors suggest that these genes may be involved in development of TDI-induced asthma.

CTNNA3 (alpha-T catenin) is a key protein of the adherence junctional complex in epithelial cells and plays an important role in cellular adherence. The function of CTNNA3 in TDI-induced asthma is not known, but it has been shown that decreased expression of CTNNA3 may lead to increased susceptibility to TDI effects and contribute to development of DA (Bernstein *et al.*, 2013). SNP CTNNA3 polymorphisms were found to be significantly associated with TDI-induced asthma in a group of 84 Korean workers compared to 263 normal controls (Kim *et al.*, 2009). The authors conducted a genome-wide association screening study and identified two genes, CTNNA1 and CTNNA3, with multi-hit association patterns. Genetic polymorphisms of CTNNA3 (SNPs rs10762058, rs7088181 and rs4378283) showed the strongest association with TDI-induced asthma with $p<0.0001$. In addition, carriers with the minor haplotype, HT2[GG] of two genetic polymorphisms (rs10762058 and rs7088181) showed significantly lower PC20 methacholine level ($p<0.0005$) and lower mRNA expression of CTNNA3 than non-carriers. This finding suggests that genetic polymorphisms of CTNNA3 confer risk of TDI-induced asthma through increased airway hyperresponsiveness to methacholine.

A Caucasian study population including 132 workers with DA (positive specific inhalation challenge), 131 symptomatic workers with a negative challenge for DA, and 147 asymptomatic workers was examined to determine if genetic variants of CTNNA3 genes are associated with increased susceptibility to DA (Bernstein *et al.*, 2013). The DA+ and DA- workers were largely exposed to HDI with some exposure to TDI and MDI, while the controls were HDI-exposed painters. The frequencies of CTNNA3 SNPs rs7088181 and rs10762058 were associated with the DA+ phenotype. Carriers of CTNNA3 minor allele homozygotes of rs7088181 and rs10762058 SNPs were 9-fold and almost 7-fold more likely to have DA, respectively, compared to the asymptomatic control workers, but not symptomatic workers with a negative challenge.

Sixty-two workers with DA and 75 diisocyanate workers negative for DA were analyzed for SNPs associated with the immune response genes IL-4RA, IL-13, and CD14 (Bernstein *et al.*, 2006). The TH_2 cytokines IL-4 and IL-13 play key roles in airway inflammation and allergic disease and SNPs of both the IL-13 and the IL-4 receptor alpha genes, as well as SNPs in the CD14 promoter region have been associated with atopy. In this study, no associations were found with individual SNPs and DA when all diisocyanate workers (TDI-, MDI- and HDI-exposed) were considered. When only HDI-exposed workers were considered (34 with DA, 62 negative for DA), associations with immune response genes and DA were found. The strongest associations were for the two-genotype variation combination IL4RA (150V) II and CD14 (C159T) CT, and the three-genotype variation combination IL4RA (150V) II, IL13 (R110Q) RR, and CD14 (C159T) CT.

Neurogenic inflammation has been found to have an important role in TDI-induced airway hyperresponsiveness. Ye *et al*. (2006) examined neurokinin 2 receptor (NK2R) gene polymorphisms for associations with TDI-induced asthma among 70 Korean workers with TDI-induced asthma, 59 Korean asymptomatic exposed controls, and 93 unexposed healthy controls. NK2R mRNA expression was higher in asthmatics than non-smoking controls. Tachykinins such as Substance P and neurokinin A, released with exposure to an irritant, exert their effects via neurokinin receptors, resulting in augmentation or decreased inflammatory, secretory or bronchoconstrictive effects. No significant differences ($p<0.05$) could be found in allele, genotype or haplotype frequencies of two SNPs of NK2R examined (7853G>A(Gly231Glu) and 11424G>A(Arg375His)) among the three groups. However, subjects with the NK2R 7853GG genotype had higher serum vascular endothelial growth factor (VEGF) levels than those with GA or AA genotype among the TDI-exposed workers (p=0.040). VEGF is an endothelial, cell-specific, mitogenic peptide that plays a critical role in the initiation and maintenance of asthma, enhances antigen sensitization, and increases vascular permeability. Thus, the NK2R G231E polymorphism in TDI-induced asthmatics appears

to result in increased serum levels of VEGF, which contributes to perpetuation of airway inflammation.

Table 19. Odds Ratio (OR), Relative Risk (RR) or *p* value for significant genotype variations associated with increased susceptibility for isocyanate-induced asthma

Reference	Odds Ratio and/or *p* value	Genetic associations for isocyanate-induced asthma
Piirila *et al*., 2001	OR=1.89 (95%CI 1.00-3.52) (no *p* given)	**GSTM1 (null)** gene lacks enzyme activity – at increased risk for DA (59 of 109 cases and 29 of 73 controls with TDI, MDI or HDI exposure)
Wikman *et al*., 2002	OR=7.77 (95%CI 1.18-51.6) (no *p* given)	**NAT1** gene polymorphism for slow acetylation – at increased risk for DA. TDI-exposed only (23 cases, 8 controls) vs fast acetylator genotype
	OR=4.53 (95%CI 1.76-11.6) *p*=0.040	**GSTM1 (null)*NAT1 slow acetylator genotype** co-presence (43 cases and 20 controls with TDI, MDI or HDI exposure) vs fast acetylator genotype
Yucesoy *et al*., 2012	OR=2.70[a] (95%CI 1.38-5.27) *p*=0.004	**SOD2 (rs4880)** superoxide dismutase single-nucleotide polymorphism (SNP) Ala→Val substitution on SOD2 gene that decreases the activity of SOD2; comparing DA+ vs DA-
	OR=6.10[a] (95%CI 1.31-28.4) *p*=0.021	**GSTP1 (rs762803)** glutathione-S-transferase SNP of unknown functional consequence; comparing DA+ vs DA-
	OR=7.34[a] (95%CI 2.04-26.5) *p*=0.002	**GSTM1*EPHX1 (rs2854450)** co-presence of glutathione-S-transferase (GSTM1) deletion and minor allele for epoxide hydrolase (EPHX1 rs2854450); comparing DA+ vs DA-
	OR=8.55[a] (95%CI 1.05-69.9) *p*=0.045	**EPHX1 (rs2740168)*EPHX1 (rs1051741)** co-presence of two EPHXs, rs2740168 variant and a variation (rs1051741) that reduces enzyme activity; comparing DA+ vs DA-
	OR=10.36[b] (95%CI 1.47-72.96) *p*=0.019	**EPHX1 (rs1051741)** epoxide hydrolase minor allele; comparing HDI-exposed DA+ vs HDI-exposed AW
	OR=6.22[b] (95%CI 1.95-19.82) *p*=0.002	**EPHX1 (rs2740171)** epoxide hydrolase SNP minor allele; comparing HDI-exposed DA+ vs HDI-exposed AW

Table 19. Odds Ratio (OR), Relative Risk (RR) or p value for significant genotype variations associated with increased susceptibility for isocyanate-induced asthma (continued)

Reference	Odds Ratio and/or *p* value	Genetic associations for isocyanate-induced asthma
Blindow *et al*., 2015	OR=6.50 (95%CI 1.10-42.1) (*p*=0.08)	**GSTT1 (null)** comparing DA+ (6 of 12) vs DA- workers (2 of 15)
	OR=5.5 (95%CI 1.05-28.9) (*p*=0.01)	**GSTM1 (null)** coupled with >20 IU/ml total IgE in patients' blood serum (11 of 15) at increased risk to develop IgE-mediated reactions compared to GSTM1 (null) and <20 IU/ml total IgE (4 of 12 patients)
Bignon *et al*., 1994	RR=9.85 *p*=0.04	**HLA DQB1*0503** carried by 7 of 56 chromosomes of patients with TDI-induced asthma (13%), 0 of 16 chromosomes of asymptomatics (0%)
	RR=9.53 *p*=0.054	**HLA DQB1*0201/0301** carried by 6 of 28 cases TDI-induced asthma (21%), 0 of 16 asymptomatics (0%)
Balboni *et al*., 1996	RR= -- *p*=0.032	**HLA DRB1*0503** carried by 9 of 30 cases (30%) vs. 0 of 12 asymptomatic controls (0%)
	RR=2.95 *p*=0.025	**HLA DRB1*0503** carried by 9 of 30 cases (30%) vs. approx. 16 of 126 healthy controls (approx. 13%)
Mapp *et al*., 2000	*P*=0.005	**HLA DQA1*0104** carried by 16 of 67 cases TDI-induced asthma (23.9%), 0 of 27 asymptomatics (0%)
	P=0.009	**HLA DQB1*0503** carried by 14 of 67 cases TDI-induced asthma (20.9%), 0 of 27 asymptomatics (0%)
	P=0.027	**HLA DQB1*0503** carried by 14 of 67 cases TDI-induced asthma (20.9%), 9 of 101 normals (8.9%)
Kim *et al*., 2006	*P*=0.001 (cases vs. asymptomatic) *P*=0.003 (cases vs. normals)	**HLA DRB1*15-DPB1*05** carried by 10.6% in cases (n=110), 0% in exposed asymptomatic worker controls (n=94), and 2.5% in unexposed normals (n=190).
Choi *et al*., 2009	TDI-OA vs. AEC[c] OR=4.43 (95%CI 1.50-13.10) *p*=0.007	**HLA DRB1*1501-DQB1*0602-DPB1*0501** carried by 16 of 84 cases (19%), 1 of 47 asymptomatic workers (2.1%), and 4 of 127 normals (4%).
	TDI-OA vs. AEC OR=2.024 (95%CI 1.14-3.59) *p*=0.016	**HLA DRB1*1501-DQB1*0602** carried by 23 of 84 cases (27.4%), 6 of 47 asymptomatic workers (12.8%), and 15 of 127 normals (11.8%).
	TDI-OA vs. AEC OR=3.127 (95%CI 1.38-7.08) *p*=0.006	**HLA DRB1*1501-DPB1*0501** carried by 17 of 84 cases (20.2%), 2 of 47 asymptomatic workers (4.3%), and 4 of 127 normals (3.1%).

Table 19. Odds Ratio (OR), Relative Risk (RR) or p value for significant genotype variations associated with increased susceptibility for isocyanate-induced asthma (continued)

Reference	Odds Ratio and/or *p* value	Genetic associations for isocyanate-induced asthma
Kim *et al*., 2009	OR=4.445 (95%CI 2.14-9.22) *p*=0.000009	**CTNNA3 (rs4378283)** – TT genotype carried by 18 of 79 cases TDI-induced asthma (22.78%) vs. 16 of 257 controls (6.23%)
	OR=4.942 (95%CI 2.33-10.47) *p*=0.000006	**CTNNA3 (rs10762058)** – GG genotype carried by 18 of 82 cases TDI-induced asthma (21.95%) vs. 14 of 260 controls (5.38%)
Kim *et al*., 2009	OR=4.894 (95%CI 2.26-10.59) *p*=0.00001	**CTNNA3 (rs7088181)** – GG genotype carried by 17 of 83 cases TDI-induced asthma (20.48%) vs. 13 of 260 controls (5.0%)
	OR=1.852 (95%CI 1.27-3.69) *p*=0.0005	**CTNNA3 (rs10762058-rs7088181)** – HT2[GG] haplotype carried by 37.2% cases vs. 24.23% controls with lower PC_{20} for methacholine
Bernstein *et al*., 2013	OR=9.05[d] (95%CI 1.69-48.54) *p*=0.01	**CTNNA3 (rs7088181)** – homozygous for SNP minor allele comparing DA+ vs AEC
	OR=6.82 (95%CI 1.82-14.88) *p*=0.002	**CTNNA3 (rs10762058)** – homozygous for SNP minor allele comparing DA+ vs AEC
Bernstein *et al*., 2006	OR=5.2 (95%CI 1.65-28.24) *p*=0.008	**IL4RA (150V) II and CD14 (C159T) CT** HDI workers with DA 39% vs 11% among DA-negative workers
	OR=6.4 (95%CI 1.57-26.12) *p*=0.01	**IL4RA (150V) II, IL13 (R110Q) RR, and CD14 (C159T) CT** HDI workers with DA 24% vs 5% among DA-negative workers
Ye *et al*., 2006	*P*=0.040	**NK2R 7853GG** genotype had higher serum VEGF levels than those with GA or AA among TDI-exposed workers, including both DA+ and AEC.

[a] DA+ vs DA-; DA-positive diisocyanate worker group compared to DA-negative diisocyanate worker group (reported respiratory symptoms but with negative specific inhalation challenge).
[b] HDI-exposed DA+ vs HDI-exposed AW; DA-positive worker group compared to asymptomatic diisocyanate-exposed worker group
[c] TDI-OA vs. AEC: workers with TDI-induced asthma vs. asymptomatic TDI-exposed control workers.
[d] DA+ vs AEC; workers with diisocyanate-asthma vs asymptomatic HDI-exposed controls

A pilot project was undertaken by Nylander-French *et al*. (2014) to identify epigenetic changes that are influenced by exposure to HDI and that are associated with levels of the urine biomarker HDA in a worker group of 20 automotive spray painters. Previous work has shown that HDI exposure can alter urine HDI biomarker levels. The study proposed use of 5-methyl cytosine (CpG) DNA methylation levels in peripheral blood mononuclear cells as epigenetic changes that modify spray-painters HDI exposure-dose relationship and indicate disparities in toxicokinetics and urine biomarker levels. The primary analysis assessed the association between urine biomarker levels and CpG methylation status adjusting for potential confounders. A significant association between DNA methylation status and biomarker levels was found for *LPHN3* (latrophilin 3), and a borderline significance for *SCARA5* (scavenger receptor class A, member 5). The *LPHN3* and *SCARA5* predicted network interactions based on CpG loci-associated genes involved lysyl oxidases, which suggests the potential to influence urine biomarker levels through fibrosis (epithelial to mesenchymal transition) affecting HDI skin permeation in addition to oxidoreductase activity affecting amine metabolism.

Any CpG that is associated with the biomarker and HDI exposure is considered to be at least a partial mediator of the effect of HDI exposure on urine HDA biomarker levels (Nylander-French *et al.*, 2014). Thirty significant CpG loci had altered methylation associated with individual HDI inhalation and skin exposure. However, neither *LPHN3* nor *SCARA5* were associated with HDI exposure suggesting only partial mediation between HDI exposure and DNA methylation mediated effects on HDA urine biomarker levels. Genetic variables, confounding individual exposures, or other unknown variables may also partially explain inter-individual variation between exposure and the biomarker of exposure.

9. Derivation of Reference Exposure Levels

Exposure to HDI monomer or HDI polyisocyanates can result in several adverse health effects depending on the level and duration of exposure. These effects include:

1) acute sensory irritation and respiratory tract inflammation,
2) asthmatic episodes in acutely exposed non-sensitized asthmatic subjects,
3) sensitization and induction of asthma in susceptible individuals following frequent repeated exposures, and
4) an accelerated decline in lung function without evidence of sensitization with long-term, repeated exposures.

The RELs derived below take into consideration these possible health effects resulting from exposure to HDI emissions. Also taken into account is potential exposure of those individuals previously sensitized to HDI polyisocyanates through occupational exposure

or some other source, but cannot unequivocally protect all sensitized individuals in the general population (see discussion below). Specifically, it is unknown how children will react to HDI isocyanate exposure early in life when the immune system is still developing. The development of asthma from exposure to isocyanates is multifactorial and it is not well understood what the mechanism for isocyanate-induced asthma is in adults, much less children. Uncertainty factors are assigned based on data gaps and the lack of knowledge regarding the relative susceptibility of infants and children compared to adults represents a substantial data gap. Lastly, HDI polyisocyanate exposure has also been associated with development of hypersensitive pneumonitis. Due to the rarity of this disease it is not known if the RELs can protect all individuals who may be susceptible.

Due to differences in both toxic potency and site of action within the respiratory tract, separate sets of REL values have been derived for HDI monomer alone and HDI polyisocyanates, which may include small amounts of HDI monomer (<1-2%). HDI monomer is mainly found in the vapor phase and appears to be more chemically reactive than HDI polyisocyanates (Pauluhn, 2015). For HDI monomer, the earliest signs of toxicity occur in the upper respiratory tract (nasal region) in animal studies. HDI polyisocyanates occur in the aerosol phase. Particle size determines where the polyisocyanate aerosol deposits in the respiratory tract. However, in animal studies the earliest signs of toxicity appear in the pulmonary region regardless of differences in the particle size distribution between studies.

The RELs for HDI monomer and polyisocyanates are intended to protect sensitive human subpopulations, which would include isocyanate industry workers that have become sensitized to these compounds. Once sensitization has occurred, brief exposure to low concentrations of HDI-based compounds may precipitate symptoms (Redlich and Karol, 2002; Redlich *et al.*, 2007). Challenge studies of sensitized workers have used exposures usually in the range of 5 to 20 ppb HDI polyisocyanates for up to two hours leading to an asthmatic response (Baur *et al.*, 1994; Lemiere *et al.*, 1996; Sastre *et al.*, 2003). However, challenge exposure of a worker sensitized to a different isocyanate (polymeric MDI) has produced an asthmatic response at well below 1 ppb (Suojalehto *et al.*, 2011).

The uncertainty in the ability to protect all sensitized individuals with the RELs can be largely addressed in a Hot Spots risk assessment scenario by estimating the number of individuals in a population that are sensitized to HDI-based polyisocyanates or other isocyanates. If the number is small (e.g., 1 to 10 per 100,000 individuals), the risk of a sensitized person being exposed to HDI polyisocyanate emissions would be negligible. Some estimates of the number of isocyanate-sensitized individuals in a population have been published.

The Surveillance of Work-related and Occupational Respiratory Disease Project (SWORD) in the United Kingdom (UK) estimated the incidence of occupational asthma seen by respiratory and occupational physicians in 1989 and 1990 (Meredith, 1993). The crude incidence of occupational asthma in the UK was reported to be 20 cases per million working persons per year. During the two years of the study, 1085 cases of occupational asthma were recorded, of which 22.2% (241 patients) were due to isocyanate exposure. Assuming 22.2% of all occupational asthma in the UK is due to isocyanates, cases of new asthma per year due to isocyanates is 4.44 per million (241/1085 × 20/mil). However, the author noted that underreported new cases of occupational asthma likely increases the incidence by at least 3-fold. Thus, occupational asthma resulting from isocyanate exposure is likely closer to 13 cases per million per year.

In addition to the incidence of occupational asthma due to all sources of exposures to diisocyanates and polyisocyanates, Meredith (1993) also reported that 68 of the total 241 isocyanate-related cases were painters during 1989-1990. Assuming these painters were sensitized to HDI polyisocyanates, about 3.8 new cases per million per year are due specifically to HDI-based painting operations (68/1085 × 20/mil × 3). Some other job categories (i.e., other manufacturing occupations) may have cases of occupational asthma resulting from HDI polyisocyanates exposure, so this number may be low.

A review of workers' compensation claims in Ontario, Canada, from 2003-2007 showed that 12 irritant- and 112 sensitizer-induced occupational asthma claims were accepted (Ribeiro *et al.*, 2014). With respect to the latter, 26.8% (30/112) were associated with diisocyanates or polyisocyanates. Of the 30 claims, the specified agent was TDI (10 cases), MDI (10 cases), HDI (8 cases), or unnamed (2 cases). Given that the population of Ontario from 2001-2006 was 11,410,046-12,160,282 (http://www.citypopulation.de/Canada-Ontario.html), the estimated prevalence of individuals in the general population who are sensitized due to occupational exposure to isocyanates is about 2.5 individuals per million [30 cases / 12 million] over a 5-year period. If it is assumed that underreporting of occupational asthma likely undercounts the number of new cases by at least 3-fold, as was estimated by Meredith (1993), the prevalence over 5 years would be 7.5 individuals per million, about one-third (2.5 individuals) of which is likely due to exposure to HDI polyisocyanates.

Although similar population estimates have not been conducted in the United States, Verschoor and Verschoor (2014) reported that in the US alone, there are approximately 280,000 workers exposed to TDI, MDI, and/or polyurethanes used in rigid foam, flexible foam, coating, adhesive, sealants and elastomer applications. Given that California accounts for approximately 12% of the US population

(http://quickfacts.census.gov/qfd/states/06000.html) and that no less than 5% of those potentially exposed to isocyanates could become sensitized at some point during their work history (Redlich *et al.*, 2007), the frequency of sensitization due to occupational isocyanate exposure would be approximately 43 individuals per million (1680/38.8 million). This calculation assumes an equal distribution of isocyanate workers within California and compared to the US as a whole.

For HDI-based isocyanates, it has been estimated that there are about 35,000 automotive refinishing facilities in the U.S., employing about 207,000 people (Sparer *et al.*, 2004; Woskie *et al.*, 2004). This would include painters with a high potential for exposure, but also include body technicians and office workers with low exposure potential. Cullen *et al*. (1996) estimated there are 125,000 auto body painters among one quarter million auto body workers in the U.S. population. Assuming again that California accounts for approximately 12% of the U.S. population and that no less than 5% of auto body painters exposed to HDI polyisocyanates develop occupational asthma, the frequency of HDI-induced asthma among auto body workers would be approximately 19 individuals per million (125,000 × 0.12 × 0.05 / 38.8 million).

The limited data suggest that the rate of potentially HDI-sensitized individuals in an industrialized society may be between 3.8 and 19 new cases per million per year. In any particular year, perhaps as many as 43 individuals per million people may be sensitized to any diisocyanate or polyisocyanates. This small at-risk population is taken into consideration in deriving the RELs.

9.1 HDI Monomer Acute Reference Exposure Level

Study	Sangha, 1984; Shiotsuka *et al*., 2006
Study population	Male and female Sprague-Dawley rats
Exposure method	Head-only HDI vapor inhalation exposure to 0, 0.005, 0.0175, 0.150, and 0.300 ppm
Exposure continuity	Single exposure (i.e., first exposure in a discontinuous 3 week exposure study)
Exposure duration	5 hr
Critical effects	Nasal epithelium lesions
LOAEL	0.12 mg/m^3 (0.018 ppm)
NOAEL	0.034 mg/m^3 (0.005 ppm)
Time-adjusted exposure	0.059 mg/m^3 (0.0086 ppm) $C^n \times t = K$, with "n" = 3
Human Equivalent Concentration	0.059 mg/m^3 (0.0086 ppm) HEC = 1 based on HDI-specific dosimetry model
LOAEL uncertainty factor (UF_L)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2
Toxicodynamic (UF_{A-d})	√10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10
Toxicodynamic (UF_{H-d})	10
Cumulative uncertainty factor	200
Reference Exposure Level	0.3 µg/m^3 (0.04 ppb)

Reference Exposure Levels (RELs) are based on the most sensitive and relevant health effects reported in the medical and toxicological literature. Acute RELs are levels at which infrequent one-hour exposures are not expected to result in adverse health effects (OEHHA, 2008). The acute REL for HDI is intended to protect 1) individuals from acute sensory irritation and respiratory tract inflammation, 2) non-sensitized asthmatics from asthmatic episodes, 3) and to some extent, those individuals that are already sensitized to HDI or other isocyanates.

A peer-reviewed investigation of sensory irritation resulting from exposure to HDI has not been conducted in human volunteers. Moreover, reports on acute animal exposures including a NOAEL for respiratory tract changes have not been published in peer-reviewed literature. Thus, a three-week discontinuous exposure study (5 hrs/day, 5 days/week) in rats is used as the key study for acute REL derivation (summarized in Section 6.3.1).

In contrast to other common diisocyanates used in industry (i.e., TDI and MDI), the physicochemical properties of HDI vapor favor its retention predominantly in the upper respiratory airways during nasal breathing, indicating that the upper respiratory system is the critical target system (Pauluhn, 2015). Rodent histopathology results support this

finding in that only equivocal findings for laryngeal and tracheal changes were found at high HDI concentrations in 3-week and 13-week studies, with no exposure-related changes found in the lung.

Lesions observed following 3-week exposure at the lowest concentration of 0.005 ppm (0.034 mg/m^3) included increased squamous metaplasia and goblet cell hyperplasia only in the anterior portions of the nose (Shiotsuka *et al.*, 2006). The authors characterized these types of changes as "subtle adaptive epithelial response[s] to injury", and thus, not a true adverse effect. However, epithelial changes including increased squamous metaplasia and goblet cell hyperplasia to the respiratory epithelium have been used by OEHHA as the basis of 8-hour/chronic RELs for acrolein, another respiratory airway irritant gas. Other subacute studies did not observe histopathological changes to nasal epithelium of rats exposed to 0.005 ppm HDI continuously for 19 days, or for up to 49 days discontinuously (i.e., 6 hrs/day) (Astroff *et al.*, 2000a; Astroff *et al.*, 2000b). More serious nasal tissue lesions, including epithelial necrosis and chronic active inflammation, were observed at the next higher concentration of 0.0175 ppm (Sangha, 1984; Shiotsuka *et al.*, 2006). Based on the multi-day exposure studies, which suggest a near-threshold response at 0.005 ppm, OEHHA chose a health-protective approach by using a single 5-hr exposure to 0.005 ppm (0.034 mg/m^3) as the point of departure (POD) for acute REL derivation.

A time extrapolation from a 5-hr exposure to 1 hr was used applying the modified Haber's Law equation $C^n \times t = K$ with a default "n" = 3 (Pauluhn, 2002; OEHHA, 2008; Pauluhn, 2014; 2015). The resulting time-adjusted POD is 0.00588 mg/m^3 for a 1-hr exposure. Haber's Law states that the product of the concentration (C) and time of exposure (t) required to produce a specific physiologic effect are equal to a constant level or severity of response (K). When "n" is not known, a modified version of Haber's equation is used for extrapolation (i.e., "n"=3) when adjusting an exposure duration of greater than 1 hr to 1 hr. This health protective approach assumes concentration is the main driver for acute effects, rather than exposure duration. The C × t studies for PMDI showed an equal dependence on changes in concentration and duration of exposure ("n" = 1) for acute effects in the pulmonary region (Pauluhn, 2002). However, a similar C × t study has not been performed for HDI.

The Human Equivalent Concentration (HEC) adjustment was based on the HDI-specific dosimetry model by Schroeter *et al*. (2013), in which wall flux of inhaled HDI, expressed in pg/cm^2-sec, is about three times greater in the nasal region of rat compared to humans. This finding implies that absorption of inhaled HDI gas to nasal epithelial tissue and lung lining fluid is three times greater in rats than in humans, and would result in a HEC adjustment factor of 3 for REL derivation. However, the greater nasal absorption rate in rats is offset by the resulting greater tracheal HDI concentration and

three times greater wall flux in human trachea and bronchial airways compared to the rat. This is a critical region of the respiratory tract for isocyanate-induced asthma. Thus, a HEC of 1 is used.

A default interspecies toxicokinetic $UF_{A\text{-}k}$ of 2 is applied to account for residual toxicokinetic differences when using the HEC adjustment. For example, Menache *et al*. (1997) have shown that, expressed as an animal-to-human ratio, the 95% confidence interval for upper respiratory surface area could change the predicted inhaled dose ratio by up to a factor of 2. A default interspecies toxicodynamic $UF_{A\text{-}d}$ of $\sqrt{10}$ is applied to account for use of key studies employing a non-primate species and the lack of data for toxicodynamic interspecies differences.

Based on toxicokinetic modeling in infants and adults that takes into account age-related ventilation rates and respiratory tract surface area, the deposition kinetics of reactive gases in the nasal and tracheobronchial regions are generally thought not to be greatly different between adults and children (Ginsberg *et al.*, 2005; OEHHA, 2008). An intraspecies toxicokinetic $UF_{A\text{-}k}$ of 1 would be indicated in this case. However, Schroeter *et al*. (2013) noted in their rat and human HDI dosimetry model extrapolations that mouth-breathing humans would increase the HDI concentration in the trachea and bronchial airways about 3-fold compared to nasal breathing. The greater nasal wall flux of inhaled HDI in rats compared to humans and the use of obligate nose-breathing rats for REL derivation points to some limitations in using rodents to model human exposure to HDI. Thus, a $UF_{A\text{-}k}$ of $\sqrt{10}$ is applied, rather than a $UF_{A\text{-}k}$ of 1, to account for potential airway regional differences in HDI deposition between nasal- and mouth-breathing humans.

An intraspecies toxicodynamic $UF_{h\text{-}d}$ is applied to address the toxicodynamic diversity in the human population, including sensitive populations. In the case of asthmagens such as HDI, OEHHA applies a $UF_{h\text{-}d}$ of 10 to the acute REL to protect children from respiratory irritation induced by inhaled HDI that may lead to an asthmatic reaction. OEHHA views asthma as a more serious health problem in children versus adults (OEHHA, 2001). The cumulative UF of 200 results in an acute REL of 0.294 $\mu g/m^3$ (0.043 ppb), which is rounded to 0.3 $\mu g/m^3$ (0.04 ppb) in the final assessment.

The toxicogenomics data for isocyanates show gene variants associated with increased toxicokinetic and toxicodynamic sensitivity up to 10-fold greater in workers developing isocyanate-induced asthma. However, these findings address long-term exposures resulting in isocyanate-induced asthma and are relevant to the 8-hour and chronic REL derivations below.

As described above, the number of potentially sensitized individuals to HDI monomer and polyisocyanates in the California population is likely very low, perhaps on the order of 3.8 to 19 new cases per million per year. The acute REL of 0.3 µg/m^3 (0.04 ppb) is below the concentration used in specific inhalation challenge studies (≥1 to 20 ppb) of potentially isocyanate-sensitized individuals, but is similar to the lowest known concentration of an isocyanate, described by Suojalehto *et al*. (2011), that resulted in an asthmatic reaction (0.05 ppb for MDI). RELs are not designed to protect every individual in a population that may be sensitized to HDI or other isocyanates (OEHHA, 2008). Additionally, the likelihood that the risk of a sensitized individual being exposed to HDI emissions from a facility is low. Thus, the acute REL is acceptable for the purposes of the Hot Spots program.

Repeated exposure to isocyanates, usually on the order of months to years, has been observed to result in sensitization in a small percentage of workers, with subsequent induction of an asthmatic state. The acute REL is designed for infrequent 1-hour exposures. There is no evidence that infrequent exposures as low as 0.3 µg/m^3 (0.04 ppb) will result in sensitization and it is unknown if this pattern of infrequent exposure can initiate and promote sensitization over time. However, retrospective studies in healthy workers frequently exposed to 0.5 to 0.78 ppb HDI have not resulted in isocyanate-induced asthma (Hathaway *et al.*, 1999; Cassidy *et al.*, 2010). Thus, the acute REL is expected to be protective against induction of sensitization for the general population with infrequent exposure.

9.2 HDI-Based Polyisocyanates Acute Reference Exposure Level

Study	Ma-Hock *et al*., 2007
Study population	Male Wistar rats
Exposure method	Nose-only inhalation of HDI-based polyisocyanate aerosol, 0, 0.5, 2.7, and 15 mg/m^3
Exposure continuity	Single exposure
Exposure duration	6 hours
Critical effects	Increased total protein in BALF
LOAEL	2.7 mg/m^3
NOAEL	0.5 mg/m^3
Modeled threshold NOAEL concentration	1.1 mg/m^3
Time-adjusted exposure	2.00 mg/m^3
Human Equivalent Concentration	0.90 mg/m^3 (2.00 x 0.45(RDDR))
LOAEL uncertainty factor (UF_L)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2
Toxicodynamic (UF_{A-d})	√10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	√10
Toxicodynamic (UF_{H-d})	10
Cumulative uncertainty factor	200
Reference Exposure Level	4.5 µg/m^3

Acute RELs are levels at which infrequent one-hour exposures are not expected to result in adverse health effects (OEHHA, 2008). Similar to the acute REL for monomeric HDI, the acute REL for HDI polyisocyanates are intended to protect 1) individuals from acute sensory irritation and pulmonary epithelium impairment and inflammation, 2) non-sensitized asthmatics from asthmatic episodes, 3) and to some extent, those individuals that are already sensitized to HDI polyisocyanates.

HDI-based polyisocyanate mixtures used in spray paint usually contain the biuret and/or isocyanurate prepolymer, both of which are aerosols. A small amount of monomeric HDI, mainly in vapor form, is also present in HDI polyisocyanates. HDI monomer vapor primarily reacts in the upper airways and does not penetrate significantly into the lung, whereas respirable HDI prepolymer aerosols reach distal airways where they have their primary effect. Consequently, the pulmonary region is the critical region of the respiratory system for HDI polyisocyanate aerosols. Evidence of pulmonary injury resulting from acute exposure includes pulmonary edema and cellular proliferation, and increased markers of inflammation in BALF. The acute REL is based on increased total protein in BALF of exposed rats, which is one of the most sensitive indicators of pulmonary epithelial changes and/or compromised function in pulmonary epithelium.

Pauluhn (2004) and Ma-Hock *et al*. (2007) exposed Wistar rats to a series of different HDI-based prepolymers/homopolymers using similar methodology protocols and found a narrow modeled NOAEL range of 1.1 to 4.1 mg/m^3 for increased total protein in BALF. The lowest of these NOAEL values (1.1 mg/m^3) was chosen as the POD for the acute REL, since there may be slight variations in toxicity among the HDI-based compounds. The NOAELs were defined by these authors with a threshold effect-type analysis using a 2 SD range above the mean of controls to define the upper bound of normal variability. OEHHA typically uses a more conservative 1 SD above the control mean for a POD in REL derivations, but the standard deviations for total protein in controls were small compared to the mean value (about 10-15% of mean values). Thus, OEHHA considered this benchmark method to be an acceptable POD for the REL.

Lee *et al*. (2003) observed a LOAEL of 1 mg/m^3 in mice for pulmonary effects resulting from acute HDI-biuret exposure. However, MMAD of the aerosol particles were 0.81 µm, smaller than the particles (1.2 to 2.3 µm) generated by Pauluhn (2004) and Ma-Hock *et al*. (2007). Studies of aerosol particles generated during paint spraying processes found that the majority of the particles are >1.5 µm (Maitre *et al.*, 1996b; Marand *et al.*, 2004). Particle size has been shown to be an important determinant for the intensity of the pulmonary irritant response (i.e., total protein in BALF) induced by polyisocyanates (Pauluhn, 2004). Thus, the studies by Pauluhn and Ma-Hock *et al*., which used aerosol particles closer to what is observed in spray painting, were considered more relevant for derivation of the acute REL.

A time extrapolation from 6 hrs to 1 hr exposure was applied using Haber's equation $C^n \times t = K$, with a default "n" = 3, when extrapolating from an exposure time greater than one hour to one hour (OEHHA, 2008). This calculation resulted in the time-adjusted NOAEL of 2.00 mg/m^3.

The Multiple Path Particle Dosimetry (MPPD) model was used to calculate the fractional deposition of HDI prepolymer aerosol into the three main regions of the respiratory tract: extrathoracic, tracheo-bronchial, and pulmonary regions (ARA, 2017). The MPPD model calculates the deposition and clearance of monodisperse and polydisperse aerosols in the respiratory tracts of rats and humans for particles ranging in size from ultrafine (0.01 µm) to coarse (20 µm). The fractional deposition for a specific lung region is included into the regional deposited dose ratio (RDDR) equation (Eq. 9-1) to determine the Human Equivalent Concentration (HEC) for REL derivation. The MPPD model represents an improvement over the US EPA (1994b) RDDR model and is now preferred for cross-species inhalation dosimetry modeling from rats to humans (U.S. EPA, 2007; Kuempel *et al.*, 2015; U.S. EPA, 2017). To obtain the modeled fractional depositions, specified input parameters are required, including particle characteristics (MADD, GSD, and density) and breathing characteristics (breathing frequency, tidal

volume, etc.) of rats and humans. The average body weight of the rats during the study period is entered into an equation to determine the minute volume of the rats (See Appendix A). The calculated rat minute volume entered into Eq. 9.1 was 187 ml/min and the standard human minute volume used was based on 20 m^3 per day air intake.

$RDDR = (SA_h / SA_r) \times (MV_r / MV_h) \times (F_r / F_h)$ **Eq. 9-1**

Where:

(r) = rat (r) and (h) = human

SA = lung surface area in cm^2 (pulmonary region)

MV = minute volume in ml/min

F = fractional deposition (pulmonary region)

Further details for MPPD model input and output parameters is provided in Appendix B. Based on the input parameters for the inhaled particles and the rat respiratory rate, the calculated RDDR was 0.45.

For potential differences between rats and humans, the default interspecies toxicokinetic UF_{A-k} of 2 is applied to account for residual toxicokinetic differences when using the HEC approach. A default interspecies toxicodynamic UF_{A-d} of $\sqrt{10}$ is applied to account for use of key studies employing non-primate species and the lack of data for toxicodynamic interspecies differences.

For the intraspecies toxicokinetic UF_{h-k}, the most sensitive effect occurs in the epithelial tissues of the pulmonary region where the relative pulmonary minute volume to surface area ratio is 3-fold greater in infants compared to adults (OEHHA, 2008). Therefore, the pulmonary effects are predicted to be greater in infants and children, resulting in a UF of $\sqrt{10}$ to account for the intra-individual variation. An intraspecies toxicodynamic uncertainty factor, UF_{h-d}, is applied to address the toxicodynamic diversity in the human population, including sensitive populations. In the case of asthmagens such as HDI-based polyisocyanates, OEHHA applies a UF_{h-d} of 10 to protect children with asthma. A cumulative UF of 200 results in an acute REL of 0.0045 mg/m^3 (4.5 µg/m^3).

The toxicogenomics data for isocyanates show gene variants associated with increased toxicokinetic and toxicodynamic sensitivity up to 10-fold greater in workers developing isocyanate-induced asthma. However, these findings address long-term exposures resulting in isocyanate-induced asthma and are relevant to the 8-hour and chronic REL derivations below.

As described in Section 9.1 the number of potentially sensitized individuals to HDI polyisocyanates in the California population is likely low (between 3.8 and 19 new cases

per million per year). Consequentially the likelihood that sensitized individuals are exposed to HDI emissions from a facility is also low. Thus, the acute REL is acceptable for the purposes of the Hot Spots program.

Repeated exposure to HDI polyisocyanates generally on the order of months to years is observed to result in sensitization to the induction of an asthmatic state in a small percentage of workers. The acute REL is designed for infrequent 1-hour exposures. There is no evidence that infrequent exposure as low as 4.5 µg/m^3 will result in sensitization or other pulmonary function deficits. Within-day and within-week decreases in lung function of spray painters have been observed following exposure to HDI polyisocyanate aerosol (Alexandersson *et al.*, 1987; Randolph *et al.*, 1997; Pourabedian *et al.*, 2010). Work day mean concentrations for HDI aerosols in spray booths resulting in short-term respiratory function changes were roughly in the range of 115 to 420 µg/m^3, which is above the acute REL of 4.5 µg/m^3. However, these exposures represent frequent, sometimes daily exposures to HDI polyisocyanates over months or years and may not reflect acute effects occurring with infrequent exposure.

Additionally, levels of actual exposures to the painters were unclear in these studies in part because of unknown or inadequate use of respiratory protection. Data from animal models suggest that the asthmogenic potency of the main polyisocyanates, HDI biuret and isocyanurate, is lower than that of HDI monomer (Pauluhn *et al.*, 2002). The lower acute REL for monomeric HDI (0.3 µg/m^3) compared to the acute REL for HDI polyisocyanates reflects this difference in toxicity. Overall, the acute REL is expected to be reasonably protective against sensitization and other pulmonary function deficits under a scenario of acute, infrequent exposures.

9.3 HDI Monomer 8-Hour Reference Exposure Level

Study	Cassidy *et al*., 2010
Study population	57 workers in HDI production (Plant 1)
Exposure method	TWA occupational exposure to HDI
Exposure continuity	6-9 hr/day (averaged to 8 hr/day), 5 days/week
Exposure duration	13.5 yrs (Plant 1)
Critical effects	HDI-induced asthma; accelerated decline in lung function
LOAEL	Not determined
NOAEL	1.23 ppb (8.46 µg/m^3), the 90th percentile of the distribution of 237 air samples
BMC_{05}	Not applicable
Time-adjusted exposure	0.88 ppb (6.044 µg/m^3)
Human equivalent concentration	Not applicable
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	1
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	10
Cumulative uncertainty factor	100
Reference Exposure Level	0.06 µg/m^3 (0.009 ppb)

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated daily 8-hour exposures, up to 7 days per week. The 8-hour REL for monomeric HDI is intended to protect individuals from 1) accelerated lung function decrements not related to HDI-induced asthma, and 2) sensitization and induction of asthma. In addition, the RELs also take into account the potential exposure of those individuals previously sensitized to HDI through occupational exposure or some other source.

The occupational study by Cassidy *et al*. (2010) used as the basis of the 8-hour REL is the same as that used for the chronic REL. The justification for using the key study is described in detail in the chronic REL derivation summary.

The only difference between the chronic REL and 8-hour REL derivation is in the time-adjusted exposure. A time-adjustment of 5 days / 7 days was applied to the 8-hour REL, since daily exposures in the critical study were 8 hours/day, 5 days/week.

9.4 HDI-Based Polyisocyanates 8-Hour Reference Exposure Level

Study	Pauluhn and Mohr, 2001
Study population	Wistar rats (10/sex/group/compound)
Exposure method	Nose-only inhalation exposure to 0, 0.4, 3 and 25 mg/m^3 HDI isocyanurate or biuret
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	13 weeks
Critical effects	Increased alveolar macrophages, thickening of septa, fibrosis, and bronchioloalveolar proliferation
LOAEL	25 mg/m^3
NOAEL	3 mg/m^3
Benchmark Concentration (BMC_{05})	Not applicable
Time-adjusted exposure	1.07 mg/m^3 (3 mg/m^3 × 6/24 × 5/7× 20/10)
Human equivalent concentration	0.90 mg/m^3 (1.07 mg/m^3 x 0.84(RDDR))
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	2
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2
Toxicodynamic (UF_{A-d})	√10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	10
Cumulative uncertainty factor	1200
Reference Exposure Level	0.8 µg/m^3 (0.0008 mg/m^3)

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated daily 8-hour exposures, up to 7 days per week. The 8-hour REL for HDI-based polyisocyanates is intended to protect individuals from 1) accelerated lung function decrements not related to HDI-induced asthma, and 2) sensitization and induction of asthma. In addition, the RELs also take into account the potential exposure of those individuals previously sensitized to HDI polyisocyanates through occupational exposure or some other source.

The subchronic animal study by Pauluhn and Mohr (2001), used as the basis of the HDI polyisocyanate 8-hour REL, is the same as that used as the basis for the chronic REL. The justification for using the key study is described in detail in the chronic REL derivation summary.

The only difference between the chronic REL and 8-hour REL derivation is in the time-adjusted exposure. A time-adjusted exposure of 6 hrs/24 hrs x 5 days/7 days x 20 m^3/10 m^3 was used for the 8-hr REL derivation, which accounts for extrapolation from the discontinuous laboratory exposure to an annualized average continuous exposure

and includes the assumption that half the daily volume of air intake in humans (i.e., 10 m^3) occurs during an active 8-hr period in accordance with OEHHA guidelines. The calculated 8-hour REL is 0.75 µg/m^3, rounded to 0.8 µg/m^3 in the final assessment.

9.5 HDI Monomer Chronic Reference Exposure Level

Study	Cassidy *et al*., 2010
Study population	57 workers in HDI production (Plant 1)
Exposure method	TWA occupational exposure to HDI
Exposure continuity	6-9 hr/day (averaged to 8 hr/day), 5 days/week
Exposure duration	13.5 yrs at (Plant 1)
Critical effects	HDI-induced asthma; accelerated decline in pulmonary lung function
LOAEL	Not determined
NOAEL	1.23 ppb (8.46 µg/m^3), the 90th percentile of the distribution of 237 air samples
$BMCL_{05}$	Not applicable
Time-adjusted exposure	0.44 ppb (3.02 µg/m^3)
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	1
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	1
Toxicodynamic (UF_{A-d})	1
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	10
Cumulative uncertainty factor	100
Reference Exposure Level	0.03 µg/m^3 (0.004 ppb)

The chronic REL is a concentration at which adverse noncancer health effects would not be expected in the general population exposed continuously (i.e., as an annualized average air concentration) over a lifetime. Analogous to the 8-hour REL for HDI, the chronic REL is intended to protect individuals from, 1) accelerated lung function decrements unrelated to HDI-induced asthma, and 2) sensitization and induction of asthma. In addition, the RELs also take into account the potential exposure of those individuals previously sensitized to HDI through occupational exposure or some other source.

Three occupational studies were found that matched exposure to HDI monomer with potential health effects. From these studies, only a free-standing NOAEL (a study NOAEL with no LOAEL) was established. In the first study, a TWA 8-hr exposure to 0.13 ppb HDI did not result in an accelerated decline in FEV_1 and FVC in 32 workers matched to controls over a 9-year period (Hathaway *et al.*, 1999). No loss of workers due to work-related respiratory problems occurred. However, examination of workers

for respiratory symptoms related to exposure was not included in the study. In the second larger study by Cassidy *et al*. (2010), a TWA 8-hour exposure to 0.78 ppb for 13.5 yrs at plant 1 (n=57) or 0.3 ppb for 9.9 yrs at plant 2 (n=43) did not result in an accelerated decline in FEV_1 and FVC. In addition, no respiratory sensitization or any other work-related respiratory problems occurred during the study. The third study by Diller *et al*. (1985) had some methodological reporting deficiencies, including an unclear number of pulmonary function measurements performed for each subject and a limited number of airborne HDI concentration estimates. However, the three studies together are supportive of the opinion that routine occupational exposure to airborne concentrations of less than 1.5 - 5.0 ppb does not result in respiratory problems in a worker population (Hathaway *et al*., 1999; Cassidy *et al*., 2010).

A POD for REL derivation was based on the study by Cassidy et al. (2010). This study provided the most comprehensive information on TWA occupational exposure to HDI in workers not wearing protective respiratory equipment. Additionally, only sample durations that approximated a full shift (about 6 to 9 hours) were included for assessment. OEHHA obtained the individual exposure data, kindly provided by Dr. Cassidy, from which a distribution was calculated and a POD selected (Table 19).

Table 19. Statistics and percentiles for TWA occupational exposure to HDI (in ppb) from Cassidy et al. (2010)

GM*	GSD	Mean**	SD	50%ile	60%ile	70%ile	75%ile	80%ile	90%ile	95%ile
0.15	4.81	0.76	3.14	0.12	0.2	0.3	0.4	0.5	1.23	2.02

*Geometric mean

**Mean based on non-detect measurements using half-LOD. If the LOD is used for non-detects in the analysis, the mean is 0.78 ppb.

The data consists of 237 personal airborne HDI samples from Plant 1, collected between 1983 and 2006 (Cassidy et al. 2010). The air monitoring data ranged from nondetectable to 31 ppb. There were 88 nondetectable samples, in which the LOD range was from 0.025 to 0.4 ppb. For the analysis, OEHHA used half the LOD for these samples. From the exposure distribution, the 90^{th} percentile was selected as the POD for REL derivation.

A freestanding NOAEL from a key study is generally not preferred for a REL derivation. However, OEHHA may use a NOAEL without an associated LOAEL identified in the same study if 1) there are no other suitable studies, and 2) the overall health hazard data (including any case reports or studies with shorter durations) are consistent with the NOAEL study (OEHHA, 2008). No other occupational or human exposure studies are currently available that have an identified LOAEL for HDI monomer. The lifetime exposure study in rats was not selected by OEHHA as the basis for the chronic REL

because 1) adequate occupational data in humans exists (in spite of the free-standing NOAEL), and 2) differences between rats and humans in regional airway deposition of inhaled HDI in modeling performed by Schroeter *et al*. (2013).

Application of a time adjustment (10/20 m^3 x 5/7 days) to the TWA 8-hour exposure (1.23 ppb) results in annualized average concentration of 0.44 ppb (3.02 µg/m^3). The time adjustment uses the default assumption that workers are inhaling half (10 m^3) their total daily air intake of 20 m^3 during their 8-hr work day.

An intraspecies toxicokinetic $UF_{H\text{-}k}$ of 10 was applied. The $UF_{H\text{-}k}$ accounts for odds ratios up to 10-fold for gene variants that resulted in isocyanate-induced asthma in workers (See Section 8). The toxicogenetic findings suggest a wide variation in response to exposure among the human population. The intraspecies toxicodynamic $UF_{H\text{-}d}$ of 10 was used to address the greater potential susceptibility of children to the asthma-exacerbating effects of HDI. The toxicogenomic data indicating associations between specific genotype and isocyanate-induced asthma (ORs between 2 and 9) for enzymes and factors related to toxicodynamic properties, including immune and inflammatory regulation, also support a UF of 10. The cumulative UF was 100, resulting in a chronic REL of 0.03 µg/m^3 (0.004 ppb).

The chronic rodent exposure study by Shiotsuka (1989) can also be evaluated as the basis of a chronic REL. However, a limitation in using this animal study is that the critical endpoint is nasal epithelial lesions rather than lung function deficits. The human database for HDI polyisocyanates and other isocyanates indicates that asthma and other types of pulmonary function deficits may be more appropriate health endpoints for chronic exposure.

9.6 HDI-Based Polyisocyanate Chronic Reference Exposure Level

Study	Pauluhn and Mohr, 2001
Study population	Wistar rats (10/sex/group/compound)
Exposure method	Nose-only inhalation exposure to 0, 0.4, 3 and 25 mg/m^3 HDI isocyanurate or biuret
Exposure continuity	6 hr/day, 5 days/week
Exposure duration	13 weeks
Critical effects	Increased alveolar macrophages, thickening of septa, fibrosis and bronchioloalveolar proliferation
LOAEL	25 mg/m^3
NOAEL	3 mg/m^3
Benchmark Concentration ($BMCL_{05}$)	Not applicable
Time-adjusted exposure	0.54 mg/m^3 (3 mg/m^3 × 6/24 × 5/7)
Human equivalent concentration	0.450 mg/m^3 (0.54 x 0.84 (RDDR))
LOAEL uncertainty factor (UF_L)	1
Subchronic uncertainty factor (UFs)	2
Interspecies uncertainty factor	
Toxicokinetic (UF_{A-k})	2
Toxicodynamic (UF_{A-d})	√10
Intraspecies uncertainty factor	
Toxicokinetic (UF_{H-k})	10
Toxicodynamic (UF_{H-d})	10
Cumulative uncertainty factor	1200
Reference Exposure Level	(0.4 µg/m^3) (0.0004 mg/m^3)

The chronic REL is a concentration at which adverse noncancer health effects would not be expected in the general population exposed continuously (i.e., as an annualized average air concentration) over a lifetime. Analogous to the 8-hour REL for HDI polyisocyanates, the chronic REL is intended to protect individuals from 1) sensitization and subsequent induction of asthma and 2) accelerated lung function decrements distinct from HDI-induced asthma. In addition, the RELs also take into account the potential exposure of those individuals previously sensitized to HDI through occupational exposure or some other source.

The POD for the chronic REL is 3 mg/m^3, the NOAEL for pulmonary changes observed in rats during a 13-week study by Pauluhn and Mohr (2001). Time adjustment from discontinuous exposure to an annualized average air concentration (6/24 hrs x 5/7 days) results in an adjusted POD of 0.536 mg/m^3. The RDDR for the isocyanurate and biuret aerosols was calculated using an MMAD = 1.4 and pulmonary fractional depositions calculated with the MPPD model, v. 3.04 (ARA, 2017). An average body weight of male (350 g) and female (218 g) rats during the 13-week study was also used in an algorithm to estimate the minute volume of the rats (Appendix A). Minute volume

of adult humans was based on the standard 20 m^3/day inhalation rate. Applying these inputs into the RDDR equation, an average RDDR value of 0.84 for males and females combined was obtained (See Eq. 9-1 and Appendix B).

For 13-week exposure studies in rodents a subchronic UF of √10 or 2 has been used by OEHHA for extrapolation to chronic exposure, depending on the findings and strength of the toxicological database. Combined with earlier acute studies with HDI isocyanurate, Pauluhn and Mohr (2001) observed that the NOAELs for both isocyanurate and biuret are in the range of 3-4 mg/m^3, whether the exposure of rats was acute, subacute, or subchronic. None of the clinical endpoints determined indicated any progression or exacerbation at any exposure concentration during the course of the exposure period. The authors also concluded that the rats appeared to adapt with continued exposure to the prepolymers over 13 weeks. Based on these findings, the lower subchronic UF of 2 is used for chronic REL derivation.

For potential differences between rats and humans, the default interspecies toxicokinetic $UF_{A\text{-}k}$ of 2 is applied to account for residual toxicokinetic differences when using the HEC approach. A default interspecies toxicodynamic $UF_{A\text{-}d}$ of √10 is applied to account for use of key studies employing non-primate species and the lack of data for toxicodynamic interspecies differences.

We assigned a value of 10 to the intraspecies toxicokinetic $UF_{H\text{-}k}$. The $UF_{H\text{-}k}$ accounts for odds ratios up to 10-fold for gene variants that resulted in isocyanate-induced asthma in workers (See Section 8). The toxicogenetic findings suggest a wide variation in response to exposure among the human population. The intraspecies $UF_{H\text{-}k}$ also accounts for the up to 3-fold greater pulmonary minute volume-to-surface area ratio in infants and children compared to adults (OEHHA, 2008).

The intraspecies toxicodynamic $UF_{H\text{-}d}$ of 10 was used to address the greater potential susceptibility of children to the asthma-exacerbating effects of HDI polyisocyanates. The toxicogenomic data indicating associations between specific genotype and isocyanate-induced asthma (ORs between 2 and 9) for enzymes and factors related to toxicodynamic properties, including immune and inflammatory regulation, also support a UF of 10. This results in a cumulative UF of 1200, resulting in a chronic REL of 0.4 $\mu g/m^3$ (0.0004 mg/m^3).

Pauluhn and Mohr (2001) concluded in their acute, subacute and subchronic animal studies that isocyanurate and biuret prepolymers had almost equivalent toxic potencies, and that any differences in toxic potency were considered related to differences in particle size. In addition, acute exposure studies carried out by Pauluhn (2004) showed that other HDI-based prepolymers had similar potencies to isocyanurate. Therefore, the

chronic REL for HDI polyisocyanates would be appropriate for HDI-based paint products containing isocyanurate and/or biuret as the main isocyanate(s). HDI polyisocyanates contain a small amount of monomeric HDI, usually less than 1%. Pauluhn (2008b) observed that the residual content of HDI monomer (0.1% vs. 2%) in two biuret aerosols produced no differences in acute pulmonary toxicity endpoints in either rats or mice. Thus, the chronic REL accounts for the residual content of monomeric HDI as long as it is at or below 2%. For any HDI-based polyisocyanates with a monomeric HDI content above 2%, it is recommended that exposure for the monomer be assessed separately. This recommendation is also applicable to the acute and 8-hour RELs.

A human chronic exposure study was not considered for derivation of the REL. Exposure to HDI-based polyisocyanates are limited to occupational studies of spray painters exposed to aerosolized paint formulations containing HDI prepolymers and small amounts of HDI monomer. Spray painters are typically hard to investigate given the usually small size of each shop, inconsistent work practices, and the sporadic nature of exposure in which brief high exposures commonly occur during spray painting operations (Redlich *et al.*, 2002). Often, poor work practices and inadequate personal respiratory protection are reasons why spray painters experience adverse pulmonary symptoms (Pisaniello and Muriale, 1989). Thus, it is difficult to estimate the concentrations the spray painters were actually breathing that led to respiratory symptoms.

Nevertheless, occupational studies support the chronic REL in that within-day, within-week and long-term declines in lung function have been observed following repeated exposure to HDI polyisocyanate aerosols over months to years (Dahlqvist *et al.*, 1995; Akbar-Khanzadeh and Rivas, 1996; Randolph *et al.*, 1997; Glindmeyer *et al.*, 2004; Pourabedian *et al.*, 2010). These accelerated reductions in lung function often appear to be distinct from HDI-induced asthma. In addition, evidence of isocyanate-induced asthma and respiratory problems have been documented in car painters during prospective studies (Redlich *et al.*, 2002; Pronk *et al.*, 2007; Dragos *et al.*, 2009). Work-day mean concentrations for HDI aerosols, mostly in the breathing zone of workers, resulted in respiratory function changes roughly in the range of 0.09 to 0.29 mg/m^3. These values are about 100- to 300-fold above the chronic REL of 0.0004 mg/m^3 and represent frequent, sometimes daily exposures to HDI polyisocyanates. However, the actual exposures were difficult to characterize, often due to unknown or inadequate use of respiratory protection.

9.7 Health Values for HDI Monomer and Polyisocyanates Derived by Other Agencies and Investigators

US EPA (1994a)

US EPA (1994a) developed an RfC for HDI monomer based on the 2-year rat study by Shiotsuka (1989). More detailed reports were published that described the basis and derivation for the RfC (Foureman *et al.*, 1994; Greenberg and Foureman, 1995). An RfC based on human data was discounted by the authors due to small cohorts, lack of mechanistic information and limited exposure data. In addition, they concluded that the human data suggest that a far greater concentration of HDI is required for sensitization than is required to elicit a reaction [in nasal tissue]. Thus, the critical endpoint chosen was nasal olfactory epithelium degeneration with a NOAEL of 0.035 mg/m^3 (0.005 ppm). The authors concluded that this endpoint was the most appropriate POD because it was the most sensitive lesion for what they considered to be an adverse effect (i.e., the lesion showed a concentration-related increase in both incidence and severity, and there was a lack of the lesion in control animals).

Some of the other lesions were considered adaptive rather than adverse, including squamous metaplasia, mucus hyperplasia, and hyaline droplet formation (Foureman *et al.*, 1994). Adaptive lesions indicate that actual functional impairment was not present [Note: OEHHA considers these types of cellular responses to a known chemical irritant to be adverse and can be used as the basis of a REL]. Even though the incidence of some of these adaptive lesions was increased at the lowest concentration, it did not increase with dose and/or time (e.g., hyaline droplet formation), which further indicated to the authors that these lesions are not appropriate to use as the basis of the RfC. Chronic inflammation was also not considered for the RfC due to the lack of concordance in incidence and severity for this lesion, which confounded the choice of a NOAEL and LOAEL.

Time adjustment from intermittent to continuous exposure (6 hr/24 hr × 5 day/7 day) resulted in a time-adjusted NOAEL of 0.006 mg/m^3. A HEC factor was then applied that accounted for differences in nasal surface area and minute volume between rats and humans (RGDR = 0.183 × 0.006 mg/m^3), resulting in an adjusted value of 0.001 mg/m^3. For uncertainty factors, an interspecies UFof 3 was applied to account for additional uncertainties when using a HEC, and an intraspecies UF of 10 was applied for sensitive human subpopulations. An additional UF of 3 was applied for lack of developmental/reproductive studies. The cumulative UF of 100 divided into the time- and HEC-adjusted NOAEL of 0.001 mg/m^3 resulted in an RfC = 1 × 10^{-5} mg/m^3 (0.01 µg/m^3, 0.0016 ppb).

Improvements in risk assessment and the HDI toxicological database have occurred since the U.S. EPA RfC was established in 1994. OEHHA notes that the RfC was derived using the NOAEL/LOAEL approach prior to the US EPA (and OEHHA) recommendation to use benchmark dose methodology, when possible, to define a low level of response for a POD. Reproductive/developmental studies for HDI (Astroff *et al.*, 2000a; Astroff *et al.*, 2000b) have been published since the RfC was derived, negating the need for a UF of 3 to account for data deficiencies in this area. In addition, more comprehensive occupational studies have been published, which OEHHA used as the basis for the chronic REL. Finally, OEHHA applied a larger intraspecies UF to account for the toxicogenetic findings that show a wide variation in response to exposure among the human population.

Pauluhn (2015)

Pauluhn (2015) proposed an occupational standard for monomeric HDI based on extensive research in a sensitized Brown Norway rat model (See Section 6.3). Using a similar protocol from a previous study with TDI, an elicitation NOAEL of 900 mg HDI/m^3 × min (131 ppm × min) was determined for rats sensitized to HDI. Using an average of 135 ppm × min for the TDI (1000 mg TDI/m^3 × min) and HDI NOAELs as a POD, Pauluhn (2015) derived an equivalent human 8-hr workplace concentration. The author divided 135 ppm × min into 480 min, then applied dosimetric adjustments of √10 for obligate vs. oronasal breathing and √10 for the assumption that humans may not depress their respiration rate and minute volume as rats do with exposure to irritant doses of TDI or HDI. An intraspecies uncertainty factor of 5 was applied based on additional sensitivity of asthmatic subjects observed in the human TDI exposure study by Baur *et al*. (1994). With these inputs, an 8-hour TLV-TWA of 0.006 ppm (0.04 mg/m^3) was calculated for workers for both HDI and TDI (135 ppm × min / (480 min × √10 × √10 × 5)). The calculated HDI value is nearly identical to the current workplace TLV-TWA standard of 0.005 ppm (NIOSH, 2015; California OSHA, 2016).

Similar to the decision not to use the US EPA RfC, OEHHA chose human occupational studies as the basis for the HDI chronic REL, rather than one based on an animal model.

Janko et al. (1992)

Currently, there are no governmental health organizations in the U.S., aside from the Oregon OSHA, with occupational health standards for HDI polyisocyanates. In 1986, the Oregon OSHA promulgated an occupational standard for HDI polyisocyanates, including HDI biuret and isocyanurate (Janko *et al.*, 1992). These limit values were manufacturer-recommended hygiene standards that were based primarily on a study in

mice by Weyel et al. (1982). In this study, HDI biuret induced a decrease in respiratory rate with a pattern indicating pulmonary irritation following an initial period of sensory irritation. The respiratory rate depression of HDI biuret was found to be six-times more potent compared to the respiratory rate depression of the pulmonary irritant gas nitrogen dioxide (NO_2). Since the TLV-TWA for NO_2 is 6 mg/m^3 and was established to prevent pulmonary irritation, a ceiling limit for HDI polyisocyanates was established at one-sixth the NO_2 value (i.e., 1 mg/m^3). In order to prevent exposures above 1 mg/m^3, a value of 0.5 mg/m^3 was designated as the 8-hr TLV-TWA.

Many studies with more sensitive indicators of pulmonary toxicity for HDI prepolymers have been published since the murine respiratory depression study. These were preferred by OEHHA to establish an acute REL value for HDI-based polyisocyanates.

9.8 Chemical as a Toxic Air Contaminant Especially Affecting Infants and Children

Under Health and Safety Code Section 39669.5, OEHHA establishes and maintains a list of Toxic Air Contaminants (TACs) that may disproportionately impact infants and children. OEHHA evaluates TACs for addition to this list as Reference Exposure Levels for TACs are developed. HDI and other isocyanates such as HDI polyisocyanates were identified by the ARB as a toxic air contaminant (TAC) in accordance with section 39657(b) of the California Health and Safety Code (Title 17, California Code of Regulations, section 93001) (CCR, 2007). HDI monomer and polyisocyanates have been shown to cause sensory irritation and respiratory tract inflammation with acute exposure in animal models, reductions in pulmonary function with chronic exposure that is distinct from HDI-induced asthma, and asthmatic reactions in HDI-sensitized workers. OEHHA considers asthma a disease that disproportionately impacts children, and thus chemicals that induce or exacerbate asthma are considered more impactful for children (OEHHA, 2001). Due to the potential of HDI-based isocyanates to exacerbate asthma and the differential impacts of asthma on children including higher prevalence rates, OEHHA recommends that HDI monomer and polyisocyanates be identified as a TAC that may disproportionally impact children pursuant to Health and Safety Code, Section 39669.5(c).

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Appendix A

Calculation of rat minute volume from rat body weight for the RELs

For the HDI polyisocyanate acute REL, Equation A-1 was used to calculate the default minute volume of rats based on mean body weight (0.262 kg). Intercept (b_0) and coefficient (b_1) values for the algorithm are from Table F.1.2 in the OEHHA Noncancer REL TSD (OEHHA, 2008):

$$\log_e(MV) = b_0 + b_1 \log_e(BW \text{ in kg}) \qquad \text{Eq. A-1}$$

where $b_0 = -0.578$, and $b_1 = 0.821$

$$\log_e(MV) = b_0 + b_1 \log_e(0.262 \text{ kg})$$

$$\log_e(MV) = -0.578 - 1.100 = -1.68$$

MV = 0.187 L/min, or 187 ml/min

For humans, an MV = 13,889 ml/min was used, based on 20 m^3 air breathed per day. This algorithm was also used to estimate minute volumes for male and female rats in the 13-week study used for the derivation of the HDI polyisocyanate 8-hour and chronic RELs.

Appendix B

MPPD modeling for the respiratory tract fractional deposition of HDI prepolymer particles, and the RDDR calculation used in the derivation of the acute REL

Input parameters based on Ma-Hock et al., (2007) study in rats exposed to HDI prepolymers.

Particle characteristics

Mean particle size for Test Substance I: 2.4 um (range: 2.2-2.6 um)
GSD for Test Substance I: 1.8 um (range: 1.7- 2.0 um)
Density: 1.04 g/cm^3 @ 25°C (for HDI monomer)
Concentration: 3.0 mg/m^3 (NOAEL for Test Substance I)
Inhalability fraction adjustment turned on

Rat Parameters

Nose only exposure selected
Minute volume: 187 ml/min (tidal volume: 1.833 ml; breaths per min: 102)

Human Parameters

Nasal breathing selected
Minute volume: 13,889 ml/min (tidal volume: 868 ml; breaths/min: 16)

MPPD Output:

Species	Fractional Deposition		
	ET	TB	PU
Human	0.5838	0.0444	0.1202
Rat (male)	0.5492	0.0335	0.0249

Regional Deposited Dose Ratio (RDDR) calculation:

Setting the same exposure concentration for the rats and humans, the RDDR is then expressed as a series of three ratios:

$$RDDR = (SA_h / SA_a) \times (MV_a / MV_h) \times (F_a / F_h)$$

Where:

SA_h = human surface area – pulmonary region (Table F.1.1, OEHHA, 2008)
SA_a = animal (rat) surface area – pulmonary region (Table F.1.1, OEHHA, 2008)
VE_a = animal (rat) minute volume
VE_h = human minute volume
F_a = animal (rat) fractional deposition – pulmonary region
F_h = human fractional deposition – pulmonary region

RDDR = (540,000 / 3400 cm^2) x (187 / 13,889 ml/min) x (0.0249 / 0.1202)
RDDR = 158.82 x 0.0136 x 0.21
RDDR = 0.45

MPPD modeling for the respiratory tract fractional deposition of HDI prepolymer particles, and the RDDR calculation used in the derivation for the 8-hour and chronic RELs

Input parameters from 13-week study in male and female Wistar rats exposed to either HDI biuret or HDI isocyanurate (Pauluhn & Mohr, 2001).

The MMAD ± GSD ranges for HDI biuret aerosol were 1.4-1.5 µm ± 1.3-1.4 for low- and mid-dose groups. The MMAD ± GSD in the high dose group was 3.3 µm ± 1.6, which was subsequently found to be less toxic by the authors. Lower toxicity was said to be due to larger particle size, which resulted in less mass of biuret reaching the pulmonary region. For isocyanurate, the MMAD ± GSD ranges were 1.4-1.5 µm ± 1.3-1.6, respectively. At the isocyanurate NOAEL of 3 mg/m^3, the MMAD ± GSD was 1.4 µm ± 1.3. The biuret NOAEL is also 3 mg/m^3 with a similar MMAD ± GSD of 1.5 µm ± 1.4. A MMAD ± GSD of 1.4 µm ± 1.3 (the NOAEL for isocyanurate) was chosen for the RDDR derivation because HDI isocyanurate particle size was more consistent between dose levels.

Particle characteristics

Mean particle size for HDI isocyanurate: 1.4 um (range: 1.4-1.5 um)
GSD for HDI isocyanurate: 1.3 um (range: 1.3-1.6 um)
Density: 1.04 g/cm^3 @ 25°C (for HDI monomer)
Concentration: 3.0 mg/m^3 (NOAEL for HDI isocyanurate)
Inhalability fraction adjustment turned on

Rat Parameters

Nose only exposure selected
Minute volume - males: 237 ml/min (tidal volume: 2.324 ml; breaths per min: 102)
Minute volume - females: 160 ml/min (tidal volume: 1.569 ml; breaths per min: 102)

Minute volumes for rats were calculated from body weights using Eq. A-1. Body weights are means during the 13 weeks of exposure.

Human Parameters

Nasal breathing selected
Minute volume: 13,889 ml/min (tidal volume: 868 ml; breaths/min: 16)

MPPD Output:

Species	Fractional Deposition		
	ET	TB	PU
Human	0.3637	0.0457	0.1222
Rat (male)	0.4673	0.0610	0.0477
Rat (female)	0.3395	0.0526	0.0420

RDDR calculation:

The same exposure concentration (3.0 mg/m^3) is set for both rats and humans. The pulmonary region (toxic endpoint) is the region applied for RDDR calculation.

$$RDDR = (SA_h / SA_a) \times (MV_a / MV_h) \times (F_a / F_h)$$

Human vs. male rats

RDDR = (540,000 / 3400 cm^2) x (237 / 13,889 ml/min) x (0.0477 / 0.1222)

RDDR = 158.82 x 0.0171 x 0.39

RDDR = 1.06

Human vs. female rats

RDDR = (540,000 / 3400 cm^2) x (160 / 13,889 ml/min) x (0.0420 / 0.1222)

RDDR = 158.82 x 0.0115 x 0.34

RDDR = 0.62

Similar pulmonary dose-responses were recorded in male and female rats exposed to HDI isocyanurate and biuret. So an average **RDDR = 0.84** is applied to the REL derivation.