

Air Toxics Hot Spots Program

Hexamethylene Diisocyanate (Monomer and Polyisocyanates) Reference Exposure Levels

Technical Support Document for the
Derivation of Noncancer Reference
Exposure Levels

Appendix D1

Public Review Draft

December 2017

Air, Community, and Environmental Research Branch

Office of Environmental Health Hazard Assessment

California Environmental Protection Agency



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Public Review Draft

Prepared by the
Office of Environmental Health Hazard Assessment

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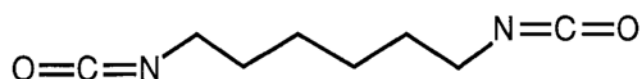
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Hexamethylene Diisocyanate Reference Exposure Levels (Monomer and Polyisocyanates)

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

CAS (monomer): 822-06-0



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 the following RELs for hexamethylene-1,6-diisocyanate (HDI) and its major prepolymers in HDI-based polyisocyanate mixtures; this document will be added to Appendix D of the TSD.

Exposure to HDI monomer gas and HDI-based polyisocyanate aerosols has been shown to cause adverse effects on the respiratory system in 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 isocyanates. Due to differences in potency and respiratory tract site of action, separate RELs have been derived for HDI monomer and HDI prepolymer mixtures. Literature summarized and referenced in this document covers the relevant published literature for HDI through summer 2017.

1.1 Hexamethylene Diisocyanate Monomer Acute REL

<i>Reference Exposure Level</i>	0.9 µg/m ³ (0.1 ppb)
<i>Critical effect(s)</i>	Nasal epithelium lesions in rodents
<i>Hazard index target(s)</i>	Respiratory system

1.2 Hexamethylene Diisocyanate Polyisocyanate Acute REL

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

1.3 Hexamethylene Diisocyanate Monomer 8-Hour REL

<i>Reference Exposure Level</i>	0.04 µg/m ³ (0.006 ppb)
<i>Critical effect(s)</i>	HDI-induced asthma; accelerated decline in lung function in humans
<i>Hazard index target(s)</i>	Respiratory system

1.4 Hexamethylene Diisocyanate Polyisocyanate 8-Hour REL

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

1.5 Hexamethylene Diisocyanate Monomer Chronic REL

<i>Reference Exposure Level</i>	0.02 µg/m ³ (0.003 ppb)
<i>Critical effect(s)</i>	HDI-induced asthma; accelerated decline in lung function in humans
<i>Hazard index target(s)</i>	Respiratory system

1.6 Hexamethylene Diisocyanate Polyisocyanate Chronic REL

<i>Reference Exposure Level</i>	0.4 µg/m ³
<i>Critical effect(s)</i>	Pulmonary inflammation and fibrosis in rodents
<i>Hazard index target(s)</i>	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	PMN	Neutrophilic granulocytes
FVC	Forced vital capacity	ppb	Parts per billion
GSD	Geometric standard deviation	ppm	Parts per million
GSH	Glutathione	RADS	Reactive airways dysfunction syndrome
GST	Glutathione-S-transferase	RD ₅₀	Concentration resulting in a 50% depression of respiratory rate
HDA	Hexamethylene diamine	RDDR	Regional deposited dose ratio
HDI	Hexamethylene diisocyanate	REL	Reference exposure level
HEC	Human equivalent concentration	RfC	Reference concentration (US EPA)
HLA	Human leucocyte antigen	RGDR	Regional gas deposition ratio
HSA	Human serum albumin	SIC	Specific inhalation challenge
IgE	Immunoglobulin E antibody type	SNP	Single nucleotide polymorphism
IgG	Immunoglobulin G antibody type	TDI	Toluene diisocyanate
LC ₀₁	Lethal concentration for 1% of animal test population	TLV	Threshold limit value
LC ₅₀	Median lethal concentration	TRIG	Total reactive isocyanate group
LDH	Lactate dehydrogenase	TSD	Technical support document
LOAEL	Lowest observed adverse effect level	TWA	Time-weighted average
MAMA	9-N-methyl-amino-anthracene	UF	Uncertainty factor
MDI	Methylene diphenyl diisocyanate	VC	Vital capacity
		VOC	Volatile organic compound

2. Physical & Chemical Properties

Sources: (SIDS, 2001; HSDB, 2016)

HDI Monomer:

<i>Description</i>	Clear colorless to slightly yellow liquid
<i>Molecular formula</i>	C ₈ H ₁₂ N ₂ O ₂
<i>Molecular weight</i>	168.20 g/mol
<i>Density</i>	1.04 g/cm ³ (25°C)
<i>Boiling point</i>	213°C
<i>Melting point</i>	-67°C
<i>Vapor pressure</i>	0.05 mm Hg (25°C); 0.7 Pa (20°C)
<i>Saturated vapor pressure</i>	30 ppm (25°C)
<i>Odor threshold</i>	Pungent; threshold 0.001 ppm
<i>Solubility</i>	Poorly soluble in water; reacts with water to form CO ₂ ; reacts with alcohols; soluble in organic solvents
<i>Conversion factor</i>	1 ppm = 6.879 mg/m ³ (25°C) 1 mg/m ³ = 0.145 ppm (25°C)

Typically, HDI monomer is industrially processed into higher molecular weight compounds with similar reactivity, but with lower volatility resulting in lower potential for exposure. Products containing these compounds still contain a residual content (<1-2%) of the HDI monomer (Fent *et al.*, 2008; Reeb-Whitaker *et al.*, 2012). Figure 1 shows the major HDI-based compounds, often referred to as prepolymers, which are commonly 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 most common HDI-based prepolymer used in polyurethane paints is the isocyanurate form, followed by biuret and uretidone. "Polyisocyanates" is another term often used to refer to a mixture of the diisocyanate monomer and various higher molecular weight diisocyanate reaction products, including 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 commonly 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. Monoisocyanates are not included in this definition. These chemically-reactive irritant compounds are not known to induce asthma.

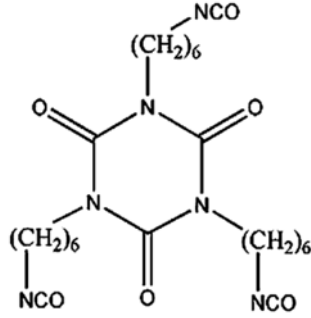
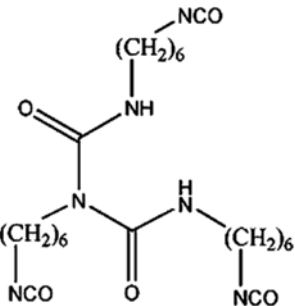
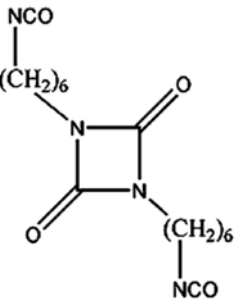
HDI Prepolymer	Properties	Chemical Structure
Isocyanurate	CAS: 3779-63-3 A ring condensate Molecular Weight: 504.7 g/mol Vapor Pressure: 5.2E-9 mmHg (20°C)	
Biuret	CAS: 4035-89-6 A condensation product of HDI and water Molecular Weight: 478.7 g/mol Vapor Pressure: 4.7E-7 mmHg (20°C)	
Uretidone	CAS: unknown 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 and its prepolymers are primarily used as hardeners for automobile and airplane polyurethane spray paints, including primers, sealers, and clear coats (Fent *et al.*, 2008). Use of HDI-based spray paints is one of the most significant occupational sources of exposure to HDI polyisocyanates (Butcher *et al.*, 1993). Other uses in paints and coatings include industrial coatings (e.g., bridges and ships) and architectural finishing (Dow Chemical Co., 2010). HDI polyisocyanates may also be found in sealants, caulking and putty compounds, adhesives, lacquers, and varnishes (SIDS, 2001).

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 from facilities that manufacture HDI monomer and HDI-based prepolymers, 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 partitioned 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 $57 \pm 9.4\%$ (Fent *et al.*, 2008). The HDI prepolymers in the overspray existed primarily in the aerosol phase.

The US Environmental Protection Agency (US EPA) (2010) reported in its Inventory Update Reporting (IUR) that the aggregated national production volume of HDI was 50 million to <100 million lbs in 2006. The California Toxics Inventory (CTI) provides emissions 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). The CTI is developed to estimate total organic gas and particulate matter emissions for area, mobile, and natural sources. Speciated emissions for each source category are then reconciled with reported stationary point source toxics data to establish a complete inventory. 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.

Some occupational health values are available for HDI monomer and HDI polyisocyanates. For monomeric HDI, the National Institute for Occupational Safety and Health (NIOSH) has a 0.005 ppm (0.035 mg/m³) time-weighted average (TWA) 8 hr exposure, and a 0.02 ppm (0.140 mg/m³) 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 (0.034 mg/m³), 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/m³ and an acceptable ceiling concentration of 1.0 mg/m³ has been developed for HDI diisocyanate-based adduct, including HDI-biuret, isocyanurate, and other polymeric forms of HDI (Oregon OSHA, 2016).

Airborne emissions of HDI monomer and prepolymers in overspray during spray paint operations are a primary source for occupational exposure. Fent et al. (2009) developed a liquid chromatography/mass spectrometry method to quantify HDI and its main prepolymers (uretidone, biuret, and isocyanurate) in samples used in the automotive refinishing industry during spray painting tasks (Table 1). Isocyanurate was the most abundant component measured in paint formulations and in air of ventilated booths during application of clear coat, although HDI polyisocyanates 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)		Paint Sampling (n=34)	
	Breathing Zone Concentration $\mu\text{g}/\text{m}^3$		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

The ranges for HDI polyisocyanates in Table 1 show that the short-term ceiling limit ($140 \mu\text{g}/\text{m}^3$) is often exceeded in the breathing zone of spray painters, demonstrating the need for acceptable respiratory protection.

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 polyisocyanates 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 (Woskie et al., 2004). IPDI would be expected to have many of the same health concerns as HDI polyisocyanates.

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 ($>1.5 \mu\text{m}$) and the gas phase, while the filter collected particles in the range of $0.01\text{-}1.5 \mu\text{m}$. In paint spray applications using HDI-based polyisocyanates, most of the spray particles were found in the impinger indicating the particles are $>1.5 \mu\text{m}$. 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 $<1.5 \mu\text{m}$.

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, very few of the particles were >10 μm aerodynamic diameter.

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, they will react slowly at room temperature 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 $\mu\text{g NCO}/\text{m}^3$, 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).

It appears reasonable to assume the HDI monomer and prepolymers will have qualitatively similar toxicities for the TRIG approach to assess overall toxicity. For example, respiratory sensitization and occupational asthma are associated with exposure to HDI in both monomeric and oligomeric forms. However, there may be differences in regional uptake in the airways, reactivity, solubility, and potency of toxic effects between the monomer and its prepolymers. In addition, the differences in toxicity are not as well understood for the prepolymers because they have not been investigated as extensively as the HDI monomer.

A study by Pauluhn (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, toluene diisocyanate (TDI) and methylene diphenyl diisocyanate (MDI) polyisocyanate mixtures even though the percent content of free NCO was 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 HDI polyisocyanates, or other polyurethane derived from it, is 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 represents an unrecognized and often unanticipated hazard (Lockey *et al.*, 2015).

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. Field measurements taken during grinding, cutting, and welding operations in car repair shops observed the release of HDI monomer concentrations up to 8 µg/m³, 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 and 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/m³ of HDI during grinding and welding operations in auto repair shops. MDI was also detected in the breathing zone of workers during these processes. Isocyanic acid and methyl isocyanate were also detected, but only during welding operations.

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.

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 highly 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 between isocyanates.

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 very 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.

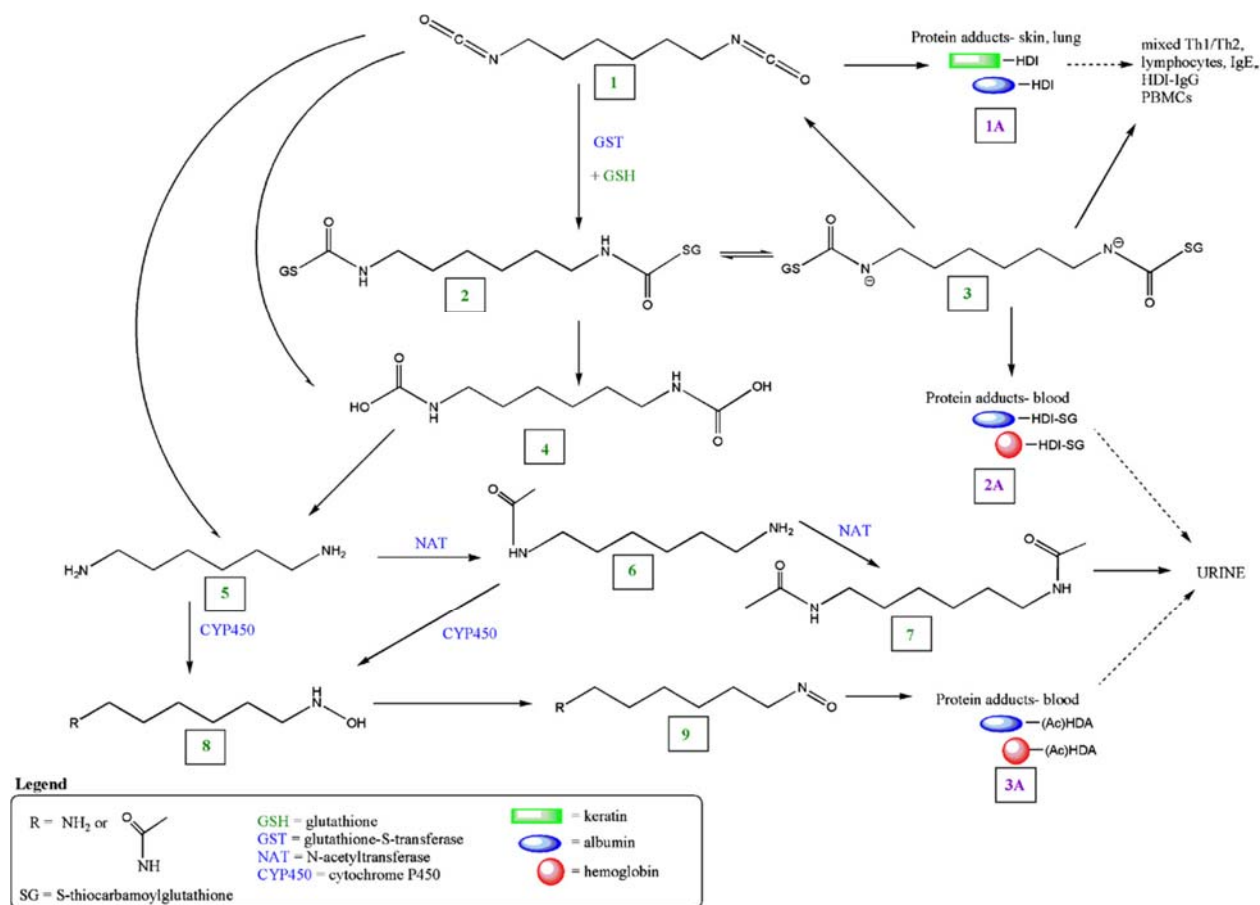


Figure 2. Proposed enzymatic (GST, NAT, CYP450) and non-enzymatic HDI metabolic pathways leading to formation of HDI–protein adducts, potential immune response, and elimination (Flack *et al.*, 2010a).

Several HDI monomer inhalation studies have been conducted in humans to estimate and quantify exposure via HDI metabolites in urine and plasma. However, only one study by Liu *et al.* (2004) investigated urinary metabolites of HDI prepolymer following inhalation exposure by 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 exposed in a chamber to HDI at concentrations of 11.9, 20.5 and 22.1 $\mu\text{g}/\text{m}^3$ (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 3 M 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 of <0.1 µg/l).

In another metabolism study, five subjects exposed to 25 µg/m³ (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/m³ (2.1 ± 3.8 ppb) and ranged from 0.30 to 97.7 µg/m³ (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 a buildup of HDI conjugates 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.

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/m³) 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 98.7 µg/m³, 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 $\mu\text{g NCO}/\text{m}^3$. Post-exposure net increases in HDA urine concentrations varied 200-fold, from 0.4 to 101 $\mu\text{g}/\text{g}$ creatinine with an average of 16.2 $\mu\text{g}/\text{g}$ creatinine. The geometric mean urinary elimination half-life 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 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 FEV₁, 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.

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). High 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 10th 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 x 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 compared to the human (Schroeter *et al.*, 2013). The cross-sectional average of wall mass flux in this anterior region was approximately 33 pg/cm²-sec in rat vs. 12 pg/cm²-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/cm²-sec) compared to the rat (approximately 0.3-0.4 pg/cm²-sec). This finding supported the authors' conclusion that HDI exposure predominantly affects the nasal airway in rats and the bronchial airways in humans.

5. Acute Toxicity of Hexamethylene Diisocyanate

5.1 Acute Toxicity to Adult Humans

Most controlled exposure studies used monomeric HDI to assess the acute noncancer health effects even though most occupational (and possible environmental exposures) are to HDI polyisocyanates which contain very low concentrations of monomeric HDI.

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 a preexisting, nonspecific bronchial hyperreactivity may induce an asthmatic attack or asthma-like symptoms. These individuals may also have increased sensitivity to the sensory irritant effects of HDI monomer and polyisocyanates. Exposure to very high levels may lead to bronchitis, bronchial spasm and pulmonary edema. Acute high levels can also cause reactive airways dysfunction syndrome (RADS), an asthma-like illness that develops after a single exposure to a high concentration of an irritating substance (Redlich and Karol, 2002). RADS is associated with airway hyperreactivity that may persist for months or years, and is likely caused by non-immunological mechanisms.

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/m³). One of three men was said to smell HDI at 5 ppb (35 µg/m³), while all three could smell HDI at 10 ppb (70 µg/m³). At 20 ppb (140 µg/m³) HDI was clearly perceptible and caused slight irritation in two of three volunteers. Exposure to 100 ppb (700 µg/m³) 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 340°F, 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 $\mu\text{g}/\text{m}^3$ (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 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 $\mu\text{g}/\text{m}^3$ (7.8 ppb) and 98.7 $\mu\text{g}/\text{m}^3$, respectively. When expressed as total reactive isocyanate group (NCO) mass in unit volume of air, the concentration was 58.2 $\mu\text{g}/\text{m}^3$ for combined monomer and oligomers. The health effects of HDI were not the focus of this study. However, spirometry was performed several times during exposure. Exposure was ended if a 15% drop in FEV_1 from baseline was recorded. None of the workers experienced an acute change in FEV_1 during exposure.

5.2 Acute Toxicity to Infants and Children

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

5.3 Acute Toxicity to Experimental Animals

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/m³, with an extrapolated LC₀₁ of 163 mg/m³. Nasal discharge, labored respiration, respiratory distress and blepharospasm (spasm of the eyelid muscle due to painful stimuli resulting in closure of the eye) were common signs in the rats for both HDI monomer and isocyanurate. 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 separate study, the 4-hour LC₅₀ recorded 24 hrs postexposure in male Swiss-Webster mice exposed to HDI biuret was 91.2 mg/m³ (Weyel *et al.*, 1982). Lung weights in exposed mice were elevated (lung weight increased by 50% at 45 mg/m³) and lethality began to occur at the concentration inducing a 20% or greater decrease in respiratory rate (approx. 40 mg/m³ biuret and above).

Pauluhn (2000) examined the pulmonary responses, concentration dependence and time course of end points in bronchoalveolar lavage fluid (BALF) of female Wistar rats (30/dose group) following nose-only 6-hr exposure to respirable HDI isocyanurate aerosol at 0, 3.9, 15.9, 54.3, or 118.1 mg/m³. Irregular and labored breathing were observed at 54.3 mg/m³ and above. Statistically significantly increased lung wet weights ($p < 0.05$) were observed at 15.9 mg/m³ and above. Pulmonary response markers in BALF included angiotensin-converting enzyme (ACE), protein levels, alkaline phosphatase, lactate dehydrogenase (LDH), glutathione and phosphatidylcholine and were assayed at 0 and 3 hours, and 1, 3, and 7 days following exposure. The most sensitive marker indicative of epithelial barrier dysfunction including impairment of the vascular endothelium was a marked concentration dependent increase of ACE and total protein in BALF, which peaked in parallel on postexposure day 1. 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/m³, 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 at all doses by 3 days postexposure.

In the same study, appreciably increased activity of LDH in BALF ($p < 0.05$), a marker of cytotoxicity, was observed at 54.3 mg/m³ and above (Pauluhn, 2000). Alkaline phosphatase levels in BALF, a marker of pneumocyte type II activity, were maximally elevated about 3-fold above control values on day 1, but no concentration-dependent increase was apparent above 15.9 mg/m³. 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/m³. 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/m³. The author also concluded that at isocyanurate concentrations of 15.9 mg/m³ 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.

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 total protein and LDH in BALF 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/m³ (Table 2). Three of the test substances included concentrations low enough to establish a NOAEL and LOAEL for increased total protein. The HDI Homopolymer (asymmetric) NOAEL and LOAEL were 0.45 and 3.29 mg/m³, respectively, the uretdione NOAEL and LOAEL were 0.56 and 2.8 mg/m³, respectively, and the HDI-based polyisocyanate (hydrophilic ester) NOAEL and LOAEL were 0.56 and 3.21 mg/m³, 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 isocyanate upper airway irritation predominating over pulmonary irritation.

Table 2. NOAELs and LOAELs in mg/m³ for various pulmonary endpoints in rats exposed acutely to HDI prepolymer 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 prepolymers (I: polymeric emulsifier modified and II: oligomeric allophanic modified) 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 $\leq 0.13\%$. The MMAD (\pm GSD) ranges for the two substances were 1.7-2.6 μm (\pm 1.7-2.0 μm). Groups of male Wistar rats were exposed to the aerosolized formulations (target means of 0.5, 3, or 15 mg/m^3 for I and II, and also 50 mg/m^3 for II only) for six hrs followed by collection of BALF at 1, 3 and 7 days post exposure. 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 HDI-based prepolymer I. For HDI-based prepolymer II, the NOAEL and LOAEL for increased total protein was 3.0 and 16.7 mg/m^3 . Using the threshold effect protocol (defined as 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 Test Substances I and II, respectively. These were considered by the authors to be similar to threshold results observed by Pauluhn (2004) for other HDI-based polyisocyanates.

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 and lung wet weight, total cell count, LDH, total protein, and γ -glutamyl transferase in BALF, and foamy appearance of cells, rats were more sensitive 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.

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 polyisocyanate 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/m³ for PMDI and 3 mg/m³ 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 than the aliphatic isocyanurate, thus giving more time for endogenous nucleophiles contained in the lung lining fluid of the airways to react with isocyanurate.

Lee et al. (2003) chamber-exposed groups of C57BL/6 mice to 1 or 10 mg/m³ 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/m³. 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/m³ exposure groups.

To compare toxic effects, Lee et al. (2003) exposed a separate group of mice to 0.36 ppm (2.5 mg/m³) HDI monomer vapor for 3 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/m³, whereas HDI-biuret-exposed rats in the 3-week exposure study by Pauluhn and Mohr (2001) observed NOAEL of 3 mg/m³.

Sangha et al. (1981) determined the RD₅₀ (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_{50s} were 0.35 and 0.17 ppm (2.4 and 1.2 mg/m³), respectively. TDI was also assessed by the authors for respiratory depression and was observed to have a similar potency compared to HDI.

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/m³ (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/m³ (10 ppm) for 30 min. Decreased tidal volumes occurred at lower concentrations with a reversal starting at around 112 mg/m³ (16 ppm). This change in tidal volume was suggestive of vapor saturation where aerosol phase HDI begins reaching the lower airways. Penetration of aerosol phase HDI reaching the pulmonary region was verified with analysis of BALF in additional rats exposed to 112 and 190 mg/m³ 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/m³.

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/m³. The MMAD and GSD for the polyisocyanate aerosol generated were 0.6 μ m 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 (>30 min) 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, HDI-biuret was concluded by the authors to have an effect on respiratory rate due to irritation of both the upper and lower respiratory tract.

The effect of 3-hr exposures to inhaled HDI biuret (0, 8, 18, 28, 33, 79, and 121 mg/m³) on respiratory rate was also investigated in groups of English short-haired guinea pigs (Ferguson *et al.*, 1987). The ventilatory response to 10% CO₂ 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% CO₂ 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 CO₂ challenge). Histopathology of lungs in guinea pigs exposed to 121 mg/m³ 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/m³) up to 11 days resulted in an adaptation to the ventilatory response to CO₂, as opposed to a cumulative effect demonstrated for TDI from a previous study by Sangha and Alarie (1979).

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/m³ HDI isocyanurate 6 hr/day, 5 days/week to examine the asthmogenic potential of the compound (Pauluhn and Mohr, 2001). The MMAD \pm GSD

range of the aerosols generated were 1.2-1.7 $\mu\text{m} \pm 1.3$ -1.7, respectively. At the end of the exposure period, body weights were significantly decreased at the highest concentration. Lung wet weights were only mildly increased ($p>0.05$), but alveolar macrophages in BALF were statistically significantly increased ($p<0.05$) at 69.2 mg/m³. 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. 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 for a single acute exposure.

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

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

Reference	Species	Exposure	Results
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	<ul style="list-style-type: none"> • 60 min RD₅₀ 0.35 ppm (2.4 mg/m³) • 180 min RD₅₀ 0.17 ppm (1.2 mg/m³)
Weyel et al., (1982)	Young adult male Swiss-Webster mice	Chamber exposure 4 hrs HDI Biuret 25 to 131 mg/m ³	<ul style="list-style-type: none"> • LC₅₀ – 91.2 mg/m³ • Lung weight increased by 50% at 45 mg/m³ • Respiratory effects indicate irritation of both upper and lower airways: initial \downarrow in respiratory rate, followed later by pause between breaths • Cannulated mice showed \downarrow respiratory rate only after 60-120 min of exposure
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	<ul style="list-style-type: none"> • Coughing and apnea at higher concentrations • Subsequent exposure to 10% CO₂ suggestive of lung restriction response to HDI biuret • Only microscopic inflammatory effects in lungs at 121 mg/m³

	Adult male English short-haired guinea pigs	3 hr exposures for 11 days HDI biuret 27.5 - 34.4 mg/m ³	<ul style="list-style-type: none"> Adaptation to ventilatory response to CO₂
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	<ul style="list-style-type: none"> HDI LC₅₀: 124 mg/m³ (18 ppm) HDI isocyanurate LC₅₀: 462 mg/m³
	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	<ul style="list-style-type: none"> ↑ ACE and total protein in BALF at 3.9 mg/m³ - most sensitive irritant response. ↑ lung wet weight at 15.9 mg/m³ ↑ LDH in BALF at 54.3 mg/m³
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	<ul style="list-style-type: none"> ↓ body weight at 69.2 mg/m³ No change in lung wet weight ↑ macrophages in BALF at 69.2 mg/m³ 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
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	<ul style="list-style-type: none"> ACE and total protein in BALF showed clear concentration-effect relationship for both isocyanates. NOEL based on above endpoints: 3 mg/m³ for HDI isocyanurate 0.5 mg/m³ for PMDI NCO content not reason for isocyanate potency differences
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 HDI monomer: nose-only 3 hrs to 2.5 mg/m ³ (0.36 ppm)	<ul style="list-style-type: none"> HDI biuret: <ul style="list-style-type: none"> 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 HDI monomer: <ul style="list-style-type: none"> No neutrophil or macrophage influx, no lung pathology. Total protein in BALF peaked at later time point.

Pauluhn (2004)	Young adult male Wistar rats	Nose-only 6 hrs HDI isocyanurate, uretidone, asymmetric HDI-homopolymer, or HDI polyisocyanate mixtures	<ul style="list-style-type: none"> · ↑ total protein in BALF ranged from 2.7 to 4.1 mg/m³ 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	<ul style="list-style-type: none"> · ↑ total protein in BALF at 1.2 to 2.6 mg/m³ 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	<ul style="list-style-type: none"> · 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, 2015	Wistar rats, age and sex not identified	Nose-only 30 min HDI monomer 4, 10, 27, 112 or 158 mg/m ³	<ul style="list-style-type: none"> · Maximum minute volume depression above 70 mg/m³ · ↓ tidal volume reversed at or above 110 mg/m³ suggesting vapor saturation and aerosol phase HDI reaching pulmonary region · ↑ total protein, PMN at 112 mg/m³ and above, and ↑ γ-glutamyl transpeptidase at 158 mg/m³

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 sensitization (asthma and asthma-like symptoms) in which a later exposure well below worker exposure limits causes an asthmatic reaction (Covestro LLC, 2015a). Symptoms following exposure of sensitized individuals include chest tightness, wheezing, cough, shortness of breath, or an asthmatic attack. 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.

The clinical manifestations and pathophysiological changes observed in isocyanate-induced asthma have a number of similarities to those in atopic asthma, including airway hyperreactivity, the presence of eosinophilic lung infiltrates, and mucus hypersecretion in airways (Del Prete *et al.*, 1993; Herrick *et al.*, 2003). However, atopy does not appear to be a risk factor for development of isocyanate-induced asthma. An asthmatic reaction resulting from HDI polyisocyanate exposure may be “early” (<1 hr after exposure), “late” (occurring 2-4 hr later), or “dual” (Redlich and Karol, 2002). Isolated early reactions are not common in isocyanate asthma, which suggests that the disease is not a strictly IgE-mediated phenomenon. Isocyanate-specific IgE antibodies are found in about 5-30% of patients with isocyanate-induced asthma, but also occasionally in asymptotically exposed workers. A similar low percentage of HDI polyisocyanate-specific IgE is found among spray painters with asthma induced by HDI polyisocyanates (Campo *et al.*, 2007). Isocyanate-specific IgG, including HDI polyisocyanate-specific IgG, is detected in a greater percentage of exposed workers (22-43%), but has generally been found to correlate with exposure rather than with asthma.

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 TH₂ T-cell response, isocyanate-induced asthma presents more of a mixed Th₁/TH₂ 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%) still 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 rather poor prognosis for those with isocyanate-induced asthma. Clinical re-examination of 91 workers indicated that FEV₁ reduction did not exceed the predicted decline over time in either smoking or nonsmoking patients. When Piirila *et al.* (2000) examined only those workers with HDI polyisocyanate-induced asthma, they found the workers used significantly less medication than workers with TDI- and MDI-induced asthma (OR: 0.412; CI: 0.229, 0.739). The authors concluded workers with HDI-induced asthma had a better outcome than those with asthma induced by TDI or MDI.

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 be fully avoided.

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 very uncommon in symptomatic patients who are still exposed to isocyanates. The use of inhalation challenge tests to subirritant 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 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 FEV₁) 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 FEV₁ >20% due to exposure was considered a positive reaction. Under these conditions, ten of the subjects had a positive reaction. Among these 10 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 diisocyanate in the 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 isocyanate species are reactive, upon or after collection, the sample is often exposed to a derivatization agent. 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 ($\leq 2 \mu\text{m}$). 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.

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 FEV₁ and FVC (Hathaway *et al.*, 1999). Average number of years exposed was 8.4 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/m³) while in the unit (roughly 2 hrs per day), and approximately 0.13 ppb (0.89 µg/m³) as an 8-hour TWA. The average daily (4-minute) peak exposure of 2.9 ppb (19.9 µg/m³), and the average daily 15-minute short-term exposure was 1.5 ppb (10.3 µg/m³). 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 4. 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 4. Annual average change in FEV₁ 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 FEV₁ or FVC occurs with TWA daily exposures of 0.5 ppb (3.4 µg/m³) HDI, and occasional peak exposure in the range of 1 to 10 ppb (6.9 to 69 µg/m³) 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 separate chemical manufacturing 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). 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/m³). The mean concentration was 0.78 ppb (5.37 µg/m³) with four samples above 10 ppb (69 µg/m³). Plant 2 had a total of 29 samples which ranged from nondetectable (6 total nondetects) to 2 ppb (14 µg/m³) with a mean level of 0.3 ppb (2.1 µg/m³).

During the study, no cohort members developed respiratory sensitization or any other work-related respiratory problems. For pulmonary function test analysis, FEV₁ and FVC were statistically 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 5).

Table 5. Annual average change in FEV₁ 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 by the authors as an unexpected result. They ruled out as confounders differences in excess weight gain, exposure to other chemicals and diisocyanates, and a pulmonary function test learning effect from newer employees. However, 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. Nevertheless, they concluded that this study provides support for the current ACGIH TLV-TWA of 5 ppb (34 µg/m³).

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/m³, 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 µg/m³), the MAK level (German Maximale Arbeitsplatz-Konzentration, or maximum workplace concentration). The wearing of respirators was then required in this instance.

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 (FEV₁, FVC and R_{aw}) indicated that the HDI group had better lung function than the control group (Table 6). 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 $\mu\text{g}/\text{m}^3$) HDI.

Table 6. Lung function measurements in HDI monomer-exposed workers and control workers (Diller *et al.*, 1985)

%FEV ₁		%FVC		R _{aw} (cmH ₂ O/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

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. Personal 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 $\mu\text{g NCO}\times\text{m}^3\times\text{hr}\times\text{mo}^{-1}$. The average time worked as a painter was 14.9 years. “Other” workers (n=290) in the industry were exposed to only to 8 $\mu\text{g NCO}\times\text{m}^3\times\text{hr}\times\text{mo}^{-1}$, 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 FEV₁, FEV₁/FVC, and 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.

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 workers were exposed to. Mean exposure levels ranged from $5.3 \mu\text{g}/\text{m}^3$ for spray paint repairers to $75 \mu\text{g}/\text{m}^3$ 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. 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. Although about 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 $115 \mu\text{g}/\text{m}^3$ biuret and $1.0 \mu\text{g}/\text{m}^3$ (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 $\mu\text{g}/\text{m}^3$ biuret were recorded.

Subjective symptoms, including eye, nose and throat irritation, were elevated in painters compared to mechanic controls ($p=0.14$), and statistically 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 FEV₁, 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 yr 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 $\mu\text{g}/\text{m}^3$ (0.2 ppb) monomeric HDI and 90 $\mu\text{g}/\text{m}^3$ 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 FEV₁, 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 at $p < 0.05$ with the number of exposure peaks (5 min exposure above 0.07 mg/m³ for HDI and 0.20 mg/m³ 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 highly exposed 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 FEV₁ 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/m³ (0.20 ppb) HDI and 0.09 mg/m³ HDI biuret. Median yearly number

of peak exposures ($>2.0 \text{ mg/m}^3$ 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 prepolymers were conducted by Akbar-Khanzadeh and Rivas (1996) in a plant that encapsulated automobile glass. 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 $10.7 \text{ } \mu\text{g/m}^3$ (1.6 ppb) HDI monomer and $90 \text{ } \mu\text{g/m}^3$ HDI prepolymer. No difference in respiratory symptoms was observed between the two groups. No reduction in pulmonary function (FEV_1 , FVC, $\text{FEV}_1\%\text{FVC}$) 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 yr. 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 prepolymers ($n=5$). 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 $\mu\text{g/m}^3$) ($n=7$).

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-exposed worker subgroups stratified by smoking habit over the 2.5-yr follow-up (Table 8). FVC also decreased significantly in all HDI-exposed workers combined, and in the smoking subgroup. No statistically significant changes were observed in the solvent-only exposed group. In the controls, $\text{FEV}_1\%\text{FVC}$ decreased significantly in smokers and all controls combined.

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

Group	n	% Changes		
		Δ FEV ₁	Δ FVC	Δ 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 FEV₁ and FVC were found among the groups. No difference in FEV₁ change was apparent between groups when stratified by smoking status, which was suggested to result from loss of study power. The modified least significant difference (Bonferroni) multiple comparison test showed that changes in FEV₁ for the HDI-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-exposed workers were significantly greater than that of the control group. Stratification by smoking status showed significant differences between the HDI-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 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 yrs (range: 1 to 30 yrs). The mean HDI polyisocyanate concentration in the spray

booths was 6.46 ± 6.64 mg/m³ (mean \pm 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 statistically significant decrease ($p=0.0002$) in mean cross-shift FEV₁ of 130.5 ± 203.19 ml was observed in the spray painters. Ten subjects (25%) had a clinically significant cross-shift decrease in FEV₁ >250 ml. None of the potential determinants of FEV₁ 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/m³) 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 FEV₁ in the spray painters.

Redlich et al. (2001)

A comprehensive cross-sectional epidemiologic study, the Study of Painters and Repairers of Autobody 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 questionnaires, week-long physiologic evaluation, blood studies, and filled out worker diaries. 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 ≥ 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 is 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. Both HDI monomer and biuret were 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 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 in Redlich et al. (2001). Among participants, 34 had stayed at the same shop, 11 that had left their original shop, and there was a 4% loss 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-ChekTM) 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 \pm 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 %	FEF ₂₅₋₇₅ %	Shortness of breath & wheeze (%)
0.5 – 5.8	66	37.0	95.0 \pm 2.1	97.3 \pm 1.7	97.3 \pm 1.0	90.2 \pm 3.8	4.6
6.1 – 14.1	58	38.3	94.5 \pm 1.6	96.3 \pm 1.5	98.1 \pm 0.8	91.0 \pm 2.9	1.7
14.2 – 20.5	56	40.7	92.5 \pm 1.7	96.7 \pm 1.8	95.7 \pm 1.2	84.1 \pm 3.5	5.4
21.1 – 57.6	60	45.4	90.6 \pm 1.7	94.0 \pm 1.7	96.3 \pm 0.8	81.5 \pm 3.5	8.3

Multiple linear regression modeling with percent predicted lung function as an outcome variable was then conducted. Expressing the results in 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 FEV₁, FEV₁/FVC and FEF₂₅₋₇₅. In addition, years spray painting was significantly associated with decreased FEV₁ and FEF₂₅₋₇₅. 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. Current use of respiratory protection was also not associated with lung function.

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.

Dragos et al. (2009)

In this prospective study of 298 apprentice car painters, 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/m³, range: 0.0006-0.006 mg/m³) while HDI oligomer levels were high (median: 0.283, range: 0.033-0.916 mg/m³). 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 ($PC_{\leq 16}$ mg/ml) were significantly associated ($p=0.01$) with the incidence of work-related lower respiratory symptoms. Associations with changes in immunoglobulins were observed at maximum values, the 95th and 97th percentile of the 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 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 pointing 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, and small particulates ≤ 2 μ m most efficiently. The mean daily TWA HDI concentration in the breathing zone was 420 ± 100 μ g/m³ (61 ± 15 ppb). Mean weekly TWA HDI exposure was lower at 130 ± 59 μ g/m³ (19 ± 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 $\geq 20\%$ 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
Hathaway et al., 1999	Nine-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
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

Study	Study type, Industry & Exposure	Findings
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	Compared to non-exposed office workers, (mean age 40 yrs): <ul style="list-style-type: none"> · 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
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	<ul style="list-style-type: none"> · 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: <ul style="list-style-type: none"> · Eye, nose and throat irritation increased in platers ($p=0.05$) but not painters. · No difference in FEV₁, 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)
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	<ul style="list-style-type: none"> · 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 FEV₁, 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	<ul style="list-style-type: none"> · 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.

Study	Study type, Industry & Exposure	Findings
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 in HDI-exposed smokers, nonsmokers and combined groups, and FVC decreased in HDI-exposed smokers and combined groups · FEV ₁ decreased significantly in HDI workers compared to solvent workers · FVC decreased significantly 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 (<i>p</i> =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 expressed as # 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 # of peak exposures associated with greater cross-week FEV ₁ decline and shortness of breath
Redlich et al., 2002	One-yr follow-up of auto shop 48 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 (<i>p</i> <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 .

Study	Study type, Industry & Exposure	Findings
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	<ul style="list-style-type: none"> · 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 IgG and IgG4 inversely related to incidence of work-related lower and upper respiratory symptoms.
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.	<ul style="list-style-type: none"> · 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 showing chronic effects 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 the respiratory symptoms of 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 62.62 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 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 FEV₁ 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 $\geq 20\%$ (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 $\geq 20\%$ were given a methacholine inhalation test. Three of these subjects had a $PC_{20} \leq 8$ 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

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/m³ for 6 hr/day, 5 days/week to examine the pulmonary and extrapulmonary effects (Pauluhn and Mohr, 2001). The MMAD \pm GSD ranges of the prepolymer aerosols generated were 1.4-2.5 $\mu\text{m} \pm$ 1.3-2.1. At study termination, lung wet weights were statistically 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/m³, and to a lesser extent at 15 mg/m³, revealed dark red, distended lungs with occasional foamy mucus in tracheas. At 75 mg/m³, 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 region (also referred to as bronchoalveolar). 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/m³.

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/m³ for 6 hr/day, 5 days/week for 13 weeks (Pauluhn and Mohr, 2001). The MMAD \pm GSD ranges for biuret aerosol were 1.4-3.3 $\mu\text{m} \pm$ 1.3-1.6 (Table 11). For isocyanurate, the MMAD \pm GSD ranges were 1.4-1.5 $\mu\text{m} \pm$ 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 11. 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/m³, including increased number of alveolar macrophages, thickening of septa, fibrosis and bronchioloalveolar proliferation (Table 12). No adverse effects were observed in nasal tissue. Overall, the toxicological responses in males and females were similar for both prepolymers.

Table 12. 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/m³, 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.

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/m³) 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 13 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 13 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 13. 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 OEHA using Fisher's Exact Test (one-tailed):

* $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

^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 observed only in the 2-week recovery animals at the two highest exposure levels, and then in nearly all male rats.

An apparent reexamination of the 3-week exposure study by Sangha (1984) 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 quantitative summary of the histopathological results was not presented. Squamous metaplasia, epithelial hyperplasia and goblet cell hyperplasia

were statistically significantly increased ($p < 0.05$) at 0.005 ppm in the nasal epithelium of the post-incisor region. However, these lesions were statistically 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 palatal ridge and the second palatal 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 palatal ridge, and second palatal ridge sections.

The persistence of epithelial cell and goblet cell hyperplasia 2 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). Olfactory epithelium degeneration did not show any substantial change in incidence or severity after 2 weeks, suggesting 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/m³), for 6 hr/day, 5 days/week (Shiotsuka, 1988). Ocular irritation, including lacrimation, was present in all rats 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 14). 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 14. 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) * $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

^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 13 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/m³) 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 observed at several intervals, suggestive of anemia, 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-yr satellite and 2-yr exposure groups was the nasal cavity (Shiotsuka, 1989; Shiotsuka *et al.*, 2010). 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 3). Chronic active inflammation showed a marked anterior to posterior gradient of incidence from the 1-yr to 2-yrs of exposure. Olfactory epithelium degeneration showed a progression of increased incidence and severity within the same anatomic region from 1- to 2-yrs 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 15. 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 15. Incidence^a and mean severity scores^b of major nasal lesions in rats exposed to monomeric HDI for 2 yrs (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 OEHA using Fisher's Exact Test (one-tailed)
* $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

^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

Other nasal lesions observed were not considered adverse (i.e., adaptive responses) by the authors or were not as sensitive an indicator of injury (Shiotsuka, 1989; Shiotsuka *et al.*, 2010). Statistically significant increases in hyperkeratosis and squamous metaplasia were observed primarily in the high dose groups. Increased incidence of epithelial hyperplasia was observed at 0.005 ppm in some nasal sections, but was quite variable and did not show a strong dose-dependence, particularly in anterior portions of the nasal cavity. Increased incidence of mucus cell hyperplasia and of hyaline droplet

degeneration was also observed in some nasal sections at the lowest exposure concentration. However, dose-dependency was often not seen and there was a lack of increased severity level with increasing dose. These lesions were also viewed by the authors as adaptive responses. Thus, 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) for evidence of a protective response to non-specific irritation.

Table 16 presents the incidence and mean severity score for what was described as the adaptive lesions resulting from 2 yr exposure to HDI (Shiotsuka, 1989). The incidence and severity of squamous metaplasia were increased only at the highest concentration in Level II males and females, and at Level V in males (data not shown). On the other hand, the incidence of epithelial hyperplasia/metaplasia was increased at the lowest two concentrations at Level I (data not shown) and Level II (Table 16), but was at or below control levels at 0.164 ppm. A similar concentration-response relationship was observed for incidence of hyaline droplet formation. The main difference is that the mean severity score for epithelial hyperplasia/metaplasia increased with increasing dose, while the mean severity score for hyaline droplet formation decreased at the high concentration, along with the incidence. Shiotsuka (1989) notes that the incidence of hyaline droplet formation was also statistically significantly increased at the lowest dose in Level IV sections (data not shown). The incidence of mucus hyperplasia was statistically significantly increased at the lowest dose at Levels II (data not shown) and III. A dose-dependent increase in incidence and severity was observed for this effect in males at Level III, and to a lesser extent in females at the same nasal level (Table 16).

Table 16. 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 yrs (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)

* $p < 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$

^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 17) 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. Analysis of the Shiotsuka (1989) original data by OEHHA suggests that little or no alveolar epithelialization 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 17. Incidence^a and mean severity scores^b of pulmonary lesions in rats exposed to monomeric HDI for 2 yrs (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)

* $p < 0.05$; ** $p \leq 0.01$

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

Foureman et al. (1994) developed a US EPA reference concentration (RfC) for HDI monomer vapor based on the chronic animal findings by Shiotsuka (1989). 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 authors used the nasal lesion findings from the chronic rat study for RfC derivation. Olfactory epithelium degeneration was chosen as the basis for the RfC because the lesion showed a progression of increased incidence and severity within the same anatomic region.

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. 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. The derivation of an RfC by Foureman et al. (1994) based on olfactory epithelial degeneration is presented in Section 9.

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. In addition, a recent report by Pauluhn (2015) 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/m³) 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 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 exposed to 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-week 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/m³ (4 ppm) HDI vapor 3 hr/day for 5 consecutive days, or by repeated intradermal injections (3×, 0.3%) on days 0, 2 and 4. For the prepolymers, groups of guinea pigs were sensitized by inhalation of the aerosol (biuret: 0, 3, 10, 48, and 142 mg/m³; isocyanurate: 0, 3, 16, 49, and 261 mg/m³) using the same protocol (5×, 3 hr/day). 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 (6×, 30%) were greater than that used for the monomer. 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 (GPSA) 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 and 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 IgG₁-antibody titer was also conducted. Monomeric HDI was found to be more potent in inducing specific IgG₁ 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 ($C \times t$) to distal airways of the lung (Pauluhn, 2015). The resulting doses needed for HDI to gain access to distal 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 $120\text{-}87\text{-}72 \text{ mg/m}^3 \times 30 \text{ min}$ 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/m³ × min (129 ppm × min).

7. Developmental and Reproductive Toxicity

Groups of 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/m³) continuously on days 0-19 of gestation (Astroff *et al.*, 2000b). Dose-dependent maternal toxicity was limited to the nasal turbinates of rats exposed to 0.05 and 0.300 ppm HDI ($p < 0.05$), including degeneration of the olfactory epithelium. No micropathological findings were observed in the nasopharynx section of the turbinates, or in the larynx, trachea, and lung. Reproductive, implantation and fetal weight endpoints were unaffected by exposure. 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/m³) (Astroff *et al.*, 2000a). Exposures were for 6 hr/day during a 14-day pre-mating 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 pre-mating phase were reduced only on Day 4. Body weights at other times, and food consumption and clinical signs were unaffected by HDI exposure during pre-mating 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 pre-mating phase (Astroff *et al.*, 2000a). 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. Organ effects were limited to microscopic lesions of the upper respiratory tract, mainly in nasal turbinates, at the two highest concentrations. 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 18), which suggests that isocyanate-induced asthma represents a complex disease phenotype determined by multiple genes. Examples of genes 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. 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 were related in this study 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 FEV₁ 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 disease. 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 18 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 18). 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 to develop 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 TH₂ 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 is 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 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 18. Variability in observed Odds Ratio (OR), Relative Risk (RR) or *p* value for significant genotype variation associations and increased susceptibility for diisocyanate-induced asthma

Reference	Odds Ratio and/or <i>p</i> value	Genetic associations for DA
Piirila et al., 2001	OR=1.89 (95%CI 1.00-3.52) (no <i>p</i> given)	GSTM1 (null) gene lacks enzyme activity (59 cases and 29 controls with TDI, MDI or HDI exposure)
Wikman et al., 2002	OR=7.77 (95%CI 1.18-51.6) (no <i>p</i> given)	NAT1 gene polymorphism for slow acetylation. TDI-exposed only (23 cases, 8 controls) vs fast acetylator genotype
	OR=4.53 (95%CI 1.76-11.6) <i>p</i> =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) <i>p</i> =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) <i>p</i> =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) <i>p</i> =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) <i>p</i> =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) <i>p</i> =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) <i>p</i> =0.002	EPHX1 (rs2740171) epoxide hydrolase SNP minor allele; comparing HDI-exposed DA+ vs HDI-exposed AW
Blindow et al., 2015	OR=6.50 (95%CI 1.10-42.1) (<i>p</i> =0.08)	GSTT1 (null) comparing DA+ (6 of 12) vs DA- workers (2 of 15)

Reference	Odds Ratio and/or <i>p</i> value	Genetic associations for DA
	OR=5.5 (95%CI 1.05-28.9) (<i>p</i> =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 <i>p</i> =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 <i>p</i> =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= -- <i>p</i> =0.032	HLA DRB1*0503 carried by 9 of 30 cases (30%) vs. 0 of 12 asymptomatic controls (0%)
	RR=2.95 <i>p</i> =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	<i>P</i> =0.005	HLA DQA1*0104 carried by 16 of 67 cases TDI-induced asthma (23.9%), 0 of 27 asymptomatics (0%)
	<i>P</i> =0.009	HLA DQB1*0503 carried by 14 of 67 cases TDI-induced asthma (20.9%), 0 of 27 asymptomatics (0%)
	<i>P</i> =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	<i>P</i> =0.001 (cases vs. asymptomatic) <i>P</i> =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) <i>p</i> =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) <i>p</i> =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) <i>p</i> =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%).
Kim et al., 2009	OR=4.445 (95%CI 2.14-9.22) <i>p</i> =0.000009	CTNNA3 (rs4378283) – TT genotype carried by 18 of 79 cases TDI-induced asthma (22.78%) vs. 16 of 257 controls (6.23%)

Reference	Odds Ratio and/or <i>p</i> value	Genetic associations for DA
	OR=4.942 (95%CI 2.33-10.47) <i>p</i> =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) <i>p</i> =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) <i>p</i> =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) <i>p</i> =0.01	CTNNA3 (rs7088181) – homozygous for SNP minor allele comparing DA+ vs AEC
	OR=6.82 (95%CI 1.82-14.88) <i>p</i> =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) <i>p</i> =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) <i>p</i> =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	<i>P</i> =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. Other variables, genetic, 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. The RELs also consider 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).

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 very small amounts of HDI monomer (usually <1%). 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). This finding suggests the HDI-based RELs may not protect all sensitized individuals from asthmatic reaction.

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 very small, 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 population 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 ($241/1085 \times 20/\text{mil}$). 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) 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 cases per million per year are due specifically to HDI-based painting operations ($68/1085 \times 20/\text{mil} \times 3$). 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-yr period. If it is assumed that underreported new cases of occupational asthma likely increase the incidence by at least 3-fold, as was estimated by Meredith (1993), the incidence 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 in California 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 ($125,000 \times 0.12 \times 0.05 / 38.8$ million).

The limited data suggest that the rate of potentially HDI-sensitized individuals in an industrialized society may be between 3.8 and 19 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 below.

9.1 HDI Monomer Acute Reference Exposure Level

<i>Study</i>	Sangha, 1984; Shiotsuka et al., 2006
<i>Study population</i>	Male and female Sprague-Dawley rats
<i>Exposure method</i>	Head-only inhalation of HDI vapor
<i>Exposure continuity</i>	5 hrs/day, 5 days/week
<i>Exposure duration</i>	3 weeks
<i>Critical effects</i>	Nasal epithelium lesions
<i>LOAEL</i>	0.120 mg/m ³ (0.0175 ppm)
<i>NOAEL</i>	0.034 mg/m ³ (0.005 ppm)
<i>Time-adjusted exposure</i>	0.17 mg/m ³ (0.025 ppm) C ⁿ × t = K with an “n” = 1
<i>Human Equivalent Concentration</i>	0.17 mg/m ³ (0.025 ppm) HEC = 1 based on HDI-specific dosimetry model
<i>LOAEL uncertainty factor (UF_L)</i>	1
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	2
<i>Toxicodynamic (UF_{A-d})</i>	√10
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	√10
<i>Toxicodynamic (UF_{H-d})</i>	10
<i>Cumulative uncertainty factor</i>	200
<i>Reference Exposure Level</i>	0.9 µg/m ³ (0.1 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 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.

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/m³) 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 these results from the 3-week exposure study, OEHHA chose a health-protective approach by using a single 5-hr exposure to 0.005 ppm as the point of departure for acute REL derivation.

Based on the $C \times t$ studies for HDI and other diisocyanates including TDI and MDI, a time extrapolation from a 5-hr exposure to 1 hr was used applying Haber’s Law $C^n \times t = K$ with an “ n ” = 1 (Pauluhn, 2002; 2014; 2015). 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). The $C \times t$ studies showed an equal dependence on changes in concentration and duration of exposure (“ n ” = 1) for acute effects on the respiratory system. Thus, the time-adjusted exposure for the NOAEL is extrapolated from 0.005 ppm for a 5-hour exposure to 0.025 ppm for a 1-hour exposure.

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/cm²-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 the region of the respiratory airway where deposition/absorption of diisocyanates and polyisocyanates may lead to sensitization. Thus, a HEC of 1 is used.

A default interspecies toxicokinetic UF_{A-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-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-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. This finding shows that mouth-breathing would bypass the critical endpoint for the acute REL (i.e., the nasal region) at the expense of increased HDI concentration in bronchial airways, the region of the lung of greatest concern for induction of asthma with repeated exposure to isocyanates. 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-k} of $\sqrt{10}$ is applied, rather than a UF_{A-k} of 1, to account for potential airway regional differences in HDI deposition between nasal- and mouth-breathing humans.

An intraspecies toxicodynamic 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, OEHHA applies a UF_{h-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 0.0009 mg/m³ (0.9 µg/m³; 0.1 ppb).

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 per million per yr. The acute REL of 0.1 ppb is below the concentration used in specific inhalation challenge studies (≥ 1 to 20 ppb) of potentially isocyanate-sensitized individuals, but is not below the lowest known concentration, described by Suojalehto *et al.* (2011), that resulted in an asthmatic reaction (0.05 ppb for MDI). RELs are not designed to protect all hypersensitive individuals in a population (OEHHA, 2008), and the likelihood that the risk of a sensitized individual being exposed to HDI emissions from a facility is very 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 0.9 µg/m³ (0.1

ppb) will result in sensitization. It is unknown if this pattern of infrequent exposure can initiate and promote sensitization over time. Retrospective studies in healthy workers frequently exposed to 0.5 to 0.78 ppb HDI for many years 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

<i>Study</i>	Ma-Hock et al., 2007
<i>Study population</i>	Male Wistar rats
<i>Exposure method</i>	Nose-only inhalation of HDI-based polyisocyanate aerosols
<i>Exposure continuity</i>	Single exposure
<i>Exposure duration</i>	6 hours
<i>Critical effects</i>	Increased total protein in bronchoalveolar lavage fluid
<i>LOAEL</i>	2.7 mg/m ³
<i>NOAEL</i>	0.5 mg/m ³
<i>Modeled threshold NOAEL concentration</i>	1.1 mg/m ³
<i>Time-adjusted exposure</i>	2.00 mg/m ³
<i>Human Equivalent Concentration</i>	0.90 mg/m ³ (2.00 x 0.45(RDDR))
<i>LOAEL uncertainty factor (UF_L)</i>	1
<i>Subchronic uncertainty factor (UFs)</i>	Not applicable
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	2
<i>Toxicodynamic (UF_{A-d})</i>	√10
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	√10
<i>Toxicodynamic (UF_{H-d})</i>	10
<i>Cumulative uncertainty factor</i>	200
<i>Reference Exposure Level</i>	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 respiratory 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 mainly contain the biuret and/or isocyanurate prepolymer, which are both aerosols. A small amount of monomeric HDI, which is a vapor, is also present in HDI polyisocyanates. HDI monomer vapor primarily reacts in the upper airways and does not penetrate significantly into the lung, whereas HDI prepolymer aerosols preferentially deposit in terminal bronchioles and alveolar

ducts. 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 of 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/m³ for increased total protein in BALF. The lowest of these NOAEL values (1.1 mg/m³) was chosen as the point of departure for the acute REL, since there may be slight variations in toxicity among the HDI-based compounds. The modeled 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 point of departure 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 point of departure for the REL.

Lee et al. (2003) observed a LOAEL of 1 mg/m³ 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 Law $C^n \times t = K$, with a default "n" = 3, when extrapolating from an exposure time greater than one hour (OEHHA, 2008). Different chemical, physical and toxicological properties of HDI polyisocyanate aerosols compared to HDI monomer (in which "n" = 1 is used) necessitates use of the default "n" = 3 when no data exists to indicate otherwise. This calculation resulted in the time-adjusted NOAEL of 2.00 mg/m³.

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 ration (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). Parameter inputs for the rat and human included the minute volume (187 ml/min for rat, 20 m³ per day for human). 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:

$$\text{RDDR} = (\text{SA}_h / \text{SA}_r) \times (\text{MV}_r / \text{MV}_h) \times (\text{F}_r / \text{F}_h) \quad \text{Eq. 9-1}$$

Where:

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

SA = lung surface area in cm² (pulmonary region)

MV = minute volume in ml/min

F = fractional deposition (pulmonary region)

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 of the lung 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/m³ (4.5 µg/m³).

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.

The number of potentially sensitized individuals to HDI polyisocyanates in the California population is likely very low (described in Section 9.1), and consequentially the

likelihood that sensitized individuals are exposed to HDI emissions from a facility is also very 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 $4.5 \mu\text{g}/\text{m}^3$ 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 100 to $450 \mu\text{g}/\text{m}^3$, which is above the acute REL of $4.5 \mu\text{g}/\text{m}^3$. 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. The data from animal models suggest that the asthmogenic potency of the main polyisocyanates, HDI biuret and isocyanurate, is minimal compared to that of HDI monomer. So the fact that the acute REL for monomeric HDI is lower ($0.9 \mu\text{g}/\text{m}^3$) than the HDI polyisocyanate acute REL is appropriate. Thus, 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

<i>Study</i>	Cassidy et al., 2010
<i>Study population</i>	100 workers in HDI production
<i>Exposure method</i>	TWA 8-hour whole-body exposure to 0.3 ppb (n=43) and 0.78 ppb (n=57) HDI
<i>Exposure continuity</i>	8 hr/day, 5 days/week
<i>Exposure duration</i>	13.5 yrs to 0.78 ppb; 9.9 yrs to 0.3 ppb
<i>Critical effects</i>	HDI-induced asthma; accelerated decline in lung function
<i>LOAEL</i>	Not determined
<i>NOAEL</i>	0.78 ppb (5.37 µg/m ³)
<i>BMC₀₅</i>	Not applicable
<i>Time-adjusted exposure</i>	0.56 ppb (0.78 × 5/7 days)
<i>Human equivalent concentration</i>	Not applicable
<i>LOAEL uncertainty factor (UF_L)</i>	1
<i>Subchronic uncertainty factor (UFs)</i>	1
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	1
<i>Toxicodynamic (UF_{A-d})</i>	1
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	10
<i>Toxicodynamic (UF_{H-d})</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Reference Exposure Level</i>	0.04 µg/m ³ (0.006 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. For the chronic REL, an additional time-adjustment factor of 10 m³ / 20 m³ is included to extrapolate to an annualized average continuous air concentration. The time adjustment for the chronic REL includes the default assumption that workers are inhaling half (10 m³) their total daily air intake of 20 m³ during their 8-hr work day.

9.4 HDI-Based Polyisocyanates 8-Hour Reference Exposure Level

<i>Study</i>	Pauluhn and Mohr, 2001
<i>Study population</i>	Wistar rats (10/sex/group/compound)
<i>Exposure method</i>	Nose-only
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	13 weeks
<i>Critical effects</i>	Increased alveolar macrophages, thickening of septa, fibrosis and bronchioloalveolar proliferation
<i>LOAEL</i>	25 mg/m ³
<i>NOAEL</i>	3 mg/m ³
<i>Benchmark Concentration (BMC₀₅)</i>	Not applicable
<i>Time-adjusted exposure</i>	1.0714 mg/m ³ (3 mg/m ³ × 6/24 × 5/7 × 20/10)
<i>Human equivalent concentration</i>	0.900 mg/m ³ (1.0714 mg/m ³ × 0.84(RDDR))
<i>LOAEL uncertainty factor (UF_L)</i>	1
<i>Subchronic uncertainty factor (UFs)</i>	2
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	2
<i>Toxicodynamic (UF_{A-d})</i>	√10
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	10
<i>Toxicodynamic (UF_{H-d})</i>	10
<i>Cumulative uncertainty factor</i>	1200
<i>Reference Exposure Level</i>	0.8 µg/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 × 5 days/7 days × 20 m³/10 m³ 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 m³) occurs during an active 8-hr period in accordance with OEHHA guidelines.

9.5 HDI Monomer Chronic Reference Exposure Level

<i>Study</i>	Cassidy et al., 2010
<i>Study population</i>	100 workers in HDI production
<i>Exposure method</i>	TWA 8-hour whole-body exposure to 0.3 ppb (n=43) and 0.78 ppb (n=57) HDI
<i>Exposure continuity</i>	8 hr/day, 5 days/week
<i>Exposure duration</i>	13.5 yrs to 0.78 ppb; 9.9 yrs to 0.3 ppb
<i>Critical effects</i>	Respiratory tract sensitization and irritation; accelerated decline in pulmonary lung function
<i>LOAEL</i>	Not determined
<i>NOAEL</i>	0.78 ppb (5.37 µg/m ³)
<i>BMCL₀₅</i>	Not applicable
<i>Time-adjusted exposure</i>	0.28 ppb (0.78 ppb × 10/20 m ³ × 5/7 d)
<i>LOAEL uncertainty factor (UF_L)</i>	1
<i>Subchronic uncertainty factor (UFs)</i>	1
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	1
<i>Toxicodynamic (UF_{A-d})</i>	1
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	10
<i>Toxicodynamic (UF_{H-d})</i>	10
<i>Cumulative uncertainty factor</i>	100
<i>Reference Exposure Level</i>	0.02 µg/m ³ (0.003 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 FEV₁ and FVC in 32 workers matched to controls over a 9-yr 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 ppm 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 FEV₁ 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 methods 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 free-standing 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 (in spite of the free-standing NOAEL), and 2) differences between rats and humans in modeled airway deposition of inhaled HDI. Work by Schroeter et al. (2013) suggests that the nasal airway lesions in rats, the most sensitive indicator of injury from exposure in this species, may not be the most sensitive airway region in humans.

Application of a time adjustment ($10/20 \text{ m}^3 \times 5/7 \text{ days}$) to the TWA 8-hour exposure (0.78 ppb) results in annualized average concentration of 0.28 ppb. 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_{a-k} of 10 was applied. The UF_{a-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-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 $0.02 \text{ } \mu\text{g}/\text{m}^3$ (0.003 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. Additionally, nasal uptake dosimetry modeling showed that wall mass flux of HDI was three times greater in rats than in humans, consequently leading to a greater concentration of HDI reaching the trachea and three times greater wall flux of HDI in bronchial airways of humans compared to rats. Finally, mouth-breathing humans would increase the concentration of HDI reaching the trachea an additional 3-fold. For acute

exposure, sensory irritation is an appropriate point of departure for REL derivation. For chronic HDI exposure, the dosimetry modeling data in rats vs. humans also support pulmonary lung function decrements and prevention of sensitization as the critical health endpoint in humans.

9.6 HDI-Based Polyisocyanate Chronic Reference Exposure Level

<i>Study</i>	Pauluhn and Mohr, 2001
<i>Study population</i>	Wistar rats (10/sex/group/compound)
<i>Exposure method</i>	Nose-only
<i>Exposure continuity</i>	6 hr/day, 5 days/week
<i>Exposure duration</i>	13 weeks
<i>Critical effects</i>	Increased alveolar macrophages, thickening of septa, fibrosis and bronchioloalveolar proliferation
<i>LOAEL</i>	25 mg/m ³
<i>NOAEL</i>	3 mg/m ³
<i>Benchmark Concentration (BMCL₀₅)</i>	Not applicable
<i>Time-adjusted exposure</i>	0.5357 mg/m ³ (3 mg/m ³ × 6/24 × 5/7)
<i>Human equivalent concentration</i>	0.450 mg/m ³ (0.5357 × 0.84 (RDDR))
<i>LOAEL uncertainty factor (UF_L)</i>	1
<i>Subchronic uncertainty factor (UFs)</i>	2
<i>Interspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{A-k})</i>	2
<i>Toxicodynamic (UF_{A-d})</i>	√10
<i>Intraspecies uncertainty factor</i>	
<i>Toxicokinetic (UF_{H-k})</i>	10
<i>Toxicodynamic (UF_{H-d})</i>	10
<i>Cumulative uncertainty factor</i>	1200
<i>Reference Exposure Level</i>	0.4 µg/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 point of departure for the chronic REL is 3 mg/m³, the NOAEL for pulmonary changes observed in rats during a 13-week study by Pauluhn and Mohr (2001). Time adjustment from discontinuous exposure (6/24 hrs × 5/7 days) to an annualized average air concentration results in an adjusted point of departure of 0.5357 mg/m³. The regional deposited dose ratio (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 approximate 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 m³/day inhalation rate. Applying these inputs into the RDDR equation, similar to that shown in Eq. 9-1 above, the average RDDR value of 0.84 for males and females combined was obtained (See Appendix B) and used in the REL derivation.

For 13-week exposure studies in rodents a subchronic UF of $\sqrt{10}$ 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/m³, 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-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.

We assigned a value of 10 to the intraspecies toxicokinetic UF_{a-k}. The UF_{a-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_{a-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-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, and a chronic REL of 0.4 µg/m³ (0.0004 mg/m³).

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 below 2%. For any HDI-based polyisocyanates with a monomeric HDI content well above 2%, it is recommended that exposure for the monomer be assessed separately.

Human chronic exposure studies 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. Work day mean concentrations for HDI aerosols in spray booths resulting in respiratory function changes were roughly in the range of 0.09 to 0.29 mg/m³. These values are about 100- to 300-fold above the chronic REL of 0.0004 mg/m³ and represent frequent, sometimes daily exposures to HDI polyisocyanates. However, the actual exposures received by the painters were unclear because of 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-yr rat study by Shiotsuka (1989). More detailed reports were published that described the basis and derivation for the RfC (Fouremant *et al.*, 1994; Greenberg and Fouremant, 1995). The critical endpoint chosen was nasal olfactory epithelium degeneration with a NOAEL of 0.035 mg/m³ (0.005 ppm). The authors concluded that this endpoint was the most appropriate for a point of departure 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). 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/m³. A HEC factor was then

applied that accounted for differences in nasal surface area and minute volume between rats and humans ($RGDR = 0.183 \times 0.006 \text{ mg/m}^3$), resulting in an adjusted value of 0.001 mg/m^3 . For uncertainty factors, an interspecies UF of 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 = $1 \times 10^{-5} \text{ mg/m}^3$ ($0.01 \text{ } \mu\text{g/m}^3$, 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 point of departure. 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 that accounted for the large genotoxic diversity in the human response to isocyanates.

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 $900 \text{ mg HDI/m}^3 \times \text{min}$ ($131 \text{ ppm} \times \text{min}$) was determined for rats sensitized to HDI. Using an average of $135 \text{ ppm} \times \text{min}$ for the TDI ($1000 \text{ mg TDI/m}^3 \times \text{min}$) and HDI NOAELs as a point of departure, Pauluhn (2015) derived an equivalent human 8-hr workplace concentration. The author divided $135 \text{ ppm} \times \text{min}$ into 480 min, then applied dosimetric adjustments of $\sqrt{10}$ for obligate vs. oronasal breathing and $\sqrt{10}$ 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 ($135 \text{ ppm} \times \text{min} / (480 \text{ min} \times \sqrt{10} \times \sqrt{10} \times 5)$). 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 (NO₂). Since the TLV-TWA for NO₂ is 6 mg/m³ and was established to prevent pulmonary irritation, a ceiling limit for HDI polyisocyanates was established at one-sixth the NO₂ value (i.e., 1 mg/m³). In order to prevent exposures above 1 mg/m³, a value of 0.5 mg/m³ 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 we develop Reference Exposure Levels for TACs. 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 disproportionately 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):

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

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

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

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

$$MV = 0.187 \text{ L/min, or } 187 \text{ ml/min}$$

For humans, an $MV = 13,889 \text{ ml/min}$ 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 μm (range: 2.2-2.6 μm)

GSD for Test Substance I: 1.8 μm (range: 1.7- 2.0 μm)

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:

$$\text{RDDR} = (\text{SA}_h / \text{SA}_a) \times (\text{MV}_a / \text{MV}_h) \times (\text{F}_a / \text{F}_h)$$

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

$$\text{RDDR} = (540,000 / 3400 \text{ cm}^2) \times (187 / 13,889 \text{ ml}/\text{min}) \times (0.0249 / 0.1202)$$

$$\text{RDDR} = 158.82 \times 0.0136 \times 0.21$$

$$\text{RDDR} = 0.45$$

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 \pm GSD ranges for HDI biuret aerosol were 1.4-1.5 $\mu\text{m} \pm 1.3$ -1.4 for low- and mid-dose groups. The MMAD \pm GSD in the high dose group was 3.3 $\mu\text{m} \pm 1.6$, 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 \pm GSD ranges were 1.4-1.5 $\mu\text{m} \pm 1.3$ -1.6, respectively. At the isocyanurate NOAEL of 3 mg/m^3 , the MMAD \pm GSD was 1.4 $\mu\text{m} \pm 1.3$. The biuret NOAEL is also 3 mg/m^3 with a similar MMAD \pm GSD of 1.5 $\mu\text{m} \pm 1.4$. A MMAD \pm GSD of 1.4 $\mu\text{m} \pm 1.3$ (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 μm (range: 1.4-1.5 μm)

GSD for HDI isocyanurate: 1.3 (range: 1.3-1.6)

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/m³) is set for both rats and humans. The pulmonary region (toxic endpoint) is the region applied for RDDR calculation.

$$\text{RDDR} = (\text{SA}_h / \text{SA}_a) \times (\text{MV}_a / \text{MV}_h) \times (\text{F}_a / \text{F}_h)$$

Human vs. male rats

$$\text{RDDR} = (540,000 / 3400 \text{ cm}^2) \times (237 / 13,889 \text{ ml/min}) \times (0.0477 / 0.1222)$$

$$\text{RDDR} = 158.82 \times 0.0171 \times 0.39$$

$$\text{RDDR} = \mathbf{1.06}$$

Human vs. female rats

$$\text{RDDR} = (540,000 / 3400 \text{ cm}^2) \times (160 / 13,889 \text{ ml/min}) \times (0.0420 / 0.1222)$$

$$\text{RDDR} = 158.82 \times 0.0115 \times 0.34$$

$$\text{RDDR} = \mathbf{0.62}$$

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.