OFFICE OF ENVIRONMENTAL HEALTH HAZARD ASSESSMENT

Air Toxics Hot Spots Program

Toluene Diisocyanate Reference Exposure Levels

Technical Support Document for the Derivation of Noncancer Reference Exposure Levels

Appendix D1

Final
March 2016

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Office of Environmental Health Hazard Assessment
California Environmental Protection Agency

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Final Report

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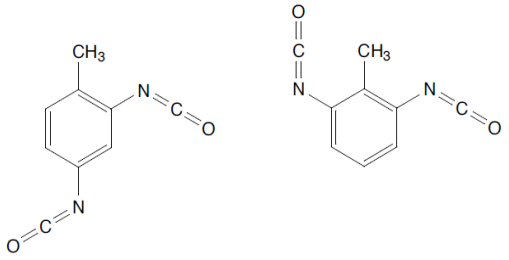
Toluene Diisocyanate Reference Exposure Levels

(2,4- and 2,6-Toluene diisocyanate, 1,3-Diisocyanatomethylbenzene, Methylphenylene isocyanate, Tolylene diisocyanate)

CAS: 26471-62-5 (mixed toluene diisocyanate isomers)



2,4-Toluene diisocyanate
CAS No. 584-84-9

2,6-Toluene diisocyanate
CAS No. 91-08-7

1. Summary

The Office of Environmental Health Hazard Assessment (OEHHA) is required to develop guidelines for conducting health risk assessments under the Air Toxics Hot Spots Program (Health and Safety Code Section 44360 (b) (2)). OEHHA developed a Technical Support Document (TSD) in response to this statutory requirement that describes acute, 8 hour and chronic RELs and was adopted in December 2008. The TSD presents methodology 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 toluene diisocyanate: this document will be added to Appendix D of the TSD.

Exposure to toluene diisocyanate (2,4- and 2,6-TDI) has been found to cause adverse effects to the respiratory system in both animals and humans. These effects include, 1) acute impacts such as sensory irritation and respiratory inflammation, 2) asthmatic episodes in non-sensitized asthmatic subjects, 3) sensitization and induction of asthma with repeated exposures, and 4) decrements in lung function due to chronic exposure without evidence of sensitization. Once asthma has been induced in TDI-sensitized individuals, triggering of attacks can occur following very low exposures (≤1 to 10 ppb) to diisocyanates. The RELs are intended to reasonably protect the general population from these health effects resulting from exposure to both 2,4- and 2,6-TDI, but may not protect all individuals previously sensitized to TDI. Literature summarized and referenced in this document covers the relevant published literature for toluene diisocyanate through Spring 2015.

1.1. Toluene Diisocyanate Acute REL

Reference Exposure Level	2 µg/m^3 (0.3 ppb)
Critical effect(s)	Asthmatic response in non-sensitized humans with asthma
Hazard index target(s)	Respiratory system

1.2. Toluene Diisocyanate 8-Hour REL

Reference Exposure Level	0.015 µg/m^3 (0.002 ppb)
Critical effect(s)	Accelerated decline in lung function; TDI-induced sensitization
Hazard index target(s)	Respiratory system

1.3. Toluene Diisocyanate Chronic REL

Reference Exposure Level	0.008 µg/m^3 (0.001 ppb)
Critical effect(s)	Accelerated decline in lung function; TDI-induced sensitization
Hazard index target(s)	Respiratory system

List of Acronyms	
Ach Acetylcholine	MEF25% Maximal expiratory flow at 25% forced vital capacity
ANOVA Analysis of variance	MV Minute volume
AW Asymptomatic exposed workers	NAT N-acetyl transferase
BAL Bronchoalveolar lavage	NDI Naphthylene diisocyanate
BL Bronchial lavage	NOAEL No observed adverse effect level
BMC Benchmark Concentration	OA Occupational asthma
BMC05 Benchmark concentration producing a 5% response rate	OR Odds Ratio
$BMCL_{05}$ the 95% lower confidence limit of the dose producing a 5% response rate	PD20 Provocation dose of methacholine (in mg) to cause a 20% drop in FEV1
BMD Benchmark Dose	PEF Peak expiratory flow
DA Diisocyanate-induced asthma	PMDI Polymeric methylene diphenyl diisocyanate
FEF25-75% Forced respiratory flow (25-75% of forced vital capacity)	PMN Neutrophilic granulocytes
FEV_1 Forced expiratory volume in 1 second	ppb Parts per billion
	ppm Parts per million
FVC Forced vital capacity	RADS Reactive airways dysfunction syndrome
FTL Ferritin light chain	RAST Radioallergosorbent test
GSH Glutathione	Raw Airway resistance
GST glutathione-S-transferase	RD50 Dose resulting in a 50% depression of respiratory rate
HDI Hexamethylene diisocyanate	REL Reference exposure level
HEC Human equivalent concentration	SGaw Specific airway conductance
HLA Human leucocyte antigen	SNP Single nucleotide polymorphism
HO-1 heme oxygenase-1	SRaw Specific airway resistance
HPLC High pressure liquid chromatography	TAC Toxic air contaminant
IPDI Isophorone diisocyanate	TDA Toluene diamine
IgE Immunoglobulin E antibody type	TDI Toluene diisocyanate
IgG Immunoglobulin G antibody type	TLV Threshold limit value
LDH Lactate dehydrogenase	TSD Technical support document
LDL Lower detection limit	TWA Time-weighted average
LOAEL Lowest observed adverse effect level	UF Uncertainty factor
MDI Methylene diphenyl diisocyanate	

2. Physical & Chemical Properties

Sources: HSDB (2015), Henschler et al. (1962), Tury et al. (2003)

Monomeric TDI Isomer	CAS	Vapor pressure
Toluene diisocyanate (mixed isomers)	26471-62-5	0.023 mm Hg 25°C
2,4-toluene diisocyanate	584-84-9	0.008 mm Hg 25°C
2,6-toluene diisocyanate	91-08-7	0.021 mm Hg 25°C

Description	Clear colorless to pale yellow liquid
Molecular formula	$C_9H_6N_2O_2$
Molecular weight	174.16 g/mol
Density	1.22 g/cm^3 (25°C)
Boiling point	251°C (mixed isomers)
Melting point	11-14°C (mixed isomers)
Saturated vapor concentration	100 mg/m^3 (14 ppm) @ 20°C
Odor threshold	>20 to 50 ppb pungent odor
Solubility	Very soluble in acetone and benzene. Sparingly soluble in water, rapidly hydrolyzes in water
Conversion factor	7.1 mg/m^3 = 1 ppm @ 25° C

3. Major Uses and Sources

Toluene diisocyanate (TDI) is used principally to make flexible polyurethane foam products, but is also used in adhesives, sealants, coatings, and elastomers (e.g., shoe soles). Commercial TDI is an isomeric mixture typically comprising 80% 2,4-toluene diisocyanate and 20% 2,6-toluene diisocyanate. Both isomeric forms are listed as Toxic Air Contaminants (TAC) (OEHHA, 2008). U.S. EPA (2010) reported in their IUR (Inventory Update Reporting) that the aggregated national production volume of TDI was 1 billion pounds or greater in 2006. Reported releases of TDI to the air in California in 2008 were at the rate of 0.28 tons/year (CARB, 2011). However, this emission level may be underestimated due to the quadrennial method of updating emission inventories in the Hot Spots program (i.e., some emitting facilities may be missing from the list for a specific year because they do not have to report emissions every year).

Given the vapor pressures of the constituent isomers, TDI released to air is expected to be in the vapor phase. Vapor-phase TDI may be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals with an estimated half-life of in the range of 3 to 24 hours (Holdren et al., 1984; Tury et al., 2003) [OEHHA notes that the toxicity of TDI reaction products has not been investigated and could be as significant as that of TDI itself]. Airborne TDI does not appear to react significantly with atmospheric water vapor and little or no measurable levels of toluene diamine (TDA), a suspected carcinogen, were found by gas phase reaction between TDI and water. Unlike the slow to insignificant reaction of airborne TDI with water vapor, the reaction of TDI added

to liquid water is relatively rapid and forms CO_2 and insoluble polyurea compounds (Yakabe et al., 1999; Schupp, 2013). Small amounts of TDA in the range of 1-2% of added TDI are also formed under static conditions. Yields of TDA increase up to 50% or greater with low loadings and increased water agitation. The studies we reviewed for developing this document did not report the presence of TDA impurities in TDI formulations. Rapid degradation of low levels of TDI in water is expected to preclude leaching or adsorption to solids in moist soils, as well as bioconcentration in aquatic organisms (Gilbert, 1988).

Occupational exposure to TDI may occur through inhalation and dermal contact during its production or use. Facility emissions of TDI are usually low due to use of closed systems during manufacturing processes. In a European government report reviewed by Tury et al. (2003), manufacture of polyurethane resulted in stack releases in the range of 0.15 to 6 mg/m^3, corresponding to an average emission of about 25 g/ton of TDI used. Other uses of TDI, as in elastomer and molded foam production, release similar or smaller amounts of TDI.

Few studies could be found that investigated exposure of residential or commercial areas to TDI emissions. Possible exposure of the general population to TDI via emissions from a U.S. facility that manufactures polyurethane foam has been reported (Darcey et al., 2002; Krone and Klingner, 2005). However, a follow-up report at five TDI manufacturing facilities in the same state show levels at one part per trillion to no current TDI exposures to nearby residents (Wilder et al., 2011).

A British case study reported TDI-induced asthma in three clerical workers exposed to TDI emissions from a neighboring polyurethane factory (Carroll et al., 1976). Exhaust from the factory was aspirated into the ventilation system of the building next door where the clerical workers were located, resulting in delayed or late-type asthmatic responses. Confirmation of TDI-induced asthma was conducted by painting a TDI-containing varnish onto a surface in a chamber exposing the workers to an estimated concentration of 1 ppb TDI. Asthmatic symptoms appeared only with exposure to varnish that contained TDI.

Dermal contact with TDI and other diisocyanates resulting in systemic sensitization has been shown in animal models, and is suspected to occur in occupational settings. The anticipated rapid degradation of emitted TDI in the atmosphere and rapid reaction of isocyanates that come into contact with surfaces would likely prevent accumulation of any TDI in aerosol form that deposits onto surfaces. Therefore, dermal exposure to TDI is not expected to be a route of exposure in the Hot Spots program.

It has also been suggested that consumers may also be exposed through use of consumer products containing TDI (Krone and Klingner, 2005). These may include products in which the monomeric or prepolymeric form of TDI is present by design, such as in paints, sealants and varnishes. However, no detectable levels of TDI have been found to be emitted from new polyurethane products

(CARB, 1996; Hugo et al., 2000; Vangronsveld et al., 2013b). Further details concerning potential exposure to TDI from consumer products are presented in Section 6.2.

Exposure in work settings in which TDI undergoes thermal degradation, such as welding, grinding, cutting, drilling, and flame lamination processes, has been reported to cause asthma and other symptoms. Burning materials that were made with diisocyanates, such as polyurethane foam in furniture, mattresses, and insulation has also been shown to release diisocyanates. Firemen or members of the general public may be exposed to diisocyanates by these sources. Thermal degradation of polyurethane foam strips 1 mm thick at temperatures of 250 to 300 °C (482 to 572 °F) resulted in emissions of TDI at 54-90 µg TDI/mg foam (Melin et al., 2001). Gas-phase TDI made up ≥93% of emissions. Increasing temperature produced an increase in the total TDI concentration and to a lesser extent TDI emissions. The proportion of TDI in the gas phase also increased with temperature.

House fires usually occur at temperatures up to 650 °C (~1200 °F). Blomqvist and colleagues (2014) characterized emissions from combustion of various household and building materials at temperatures of approximately 810 °K (538 °C, or 1000 °F). At higher temperatures, TDI breaks down more completely into isocyanic acid and other simpler products. Polyurethane mattress material was ignited in two large indoor room scenarios (43-86 m^3) and allowed to burn for up to 9-12 minutes before extinguishing with water. Isocyanic acid was the primary product released and found in indoor air (5.5 to 44 ppb), with small amounts of 2,4-TDI (0.3 to 0.8 ppb), 2,6-TDI (0.3 to 0.9 ppb) and several aminoisocyanates [OEHHA notes that, although not a sensitizer, isocyanic acid is also highly toxic].

Typically, a mixture of the toluene diisocyanate isomers (i.e., 2,4- and 2,6-TDI) is used in the production of polyurethane foams (EPA, 2015). However, TDI prepolymer use makes up a significant fraction of the production of some foams, spray lacquers, and other coatings (Vandenplas et al., 1992; Butcher et al., 1993; Bayer MaterialScience, 2005). Table 1 lists some TDI prepolymers of commercial importance.

Table 1 Toluene diisocyanate prepolymers (U. S. EPA, 2015)

Chemical Name	Chemical abstracts index name	CAS Number
Toluene diisocyanate trimer	Benzene, 1,3-diisocyanatomethyl-, trimer	9019-85-6
Poly(toluene diisocyanate)	Benzene, 1,3-diisocyanatomethyl-, homopolymer	9017-01-0
Toluene diisocyanate dimer	1,3-Diazetidine-2,4-dione, 1,3bis(3-isocyanatomethylphenyl)-	26747-90-0
Toluene diisocyanate “cyclic” trimer	1,3,5-Triazine-2,4,6(1H,3H,5H)trione, 1,3,5-tris(3isocyanatomethylphenyl)-	26603-40-7

TDI manufactured as a prepolymer (Figure 1) is much less volatile but still retains a high level of reactivity. Thus, while the potential for vapor exposure is reduced with the prepolymer, exposure to aerosols generated during use remains a possibility, as does the potential for pulmonary effects similar to that caused by monomeric TDI. A small percentage of TDI monomer is usually present in prepolymer formulations.

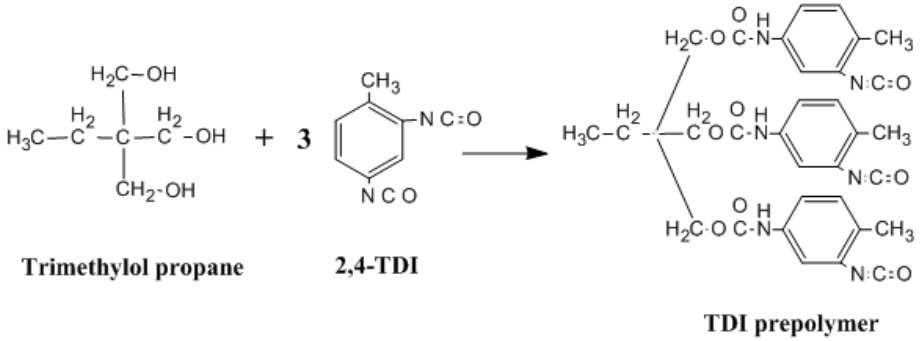


Figure 1. Formation of TDI Prepolymer

There are very few peer-reviewed toxicity studies for TDI prepolymers, which is likely a reflection of their more recent use commercially compared to monomeric TDI. However, these studies show that TDI monomer and prepolymers share many of the same pulmonary effects, including inducing sensitization and occupational asthma with repeated exposure. This suggests some commonality in the mechanisms of sensitization, possibly related to N=C=O binding carrier proteins (Bello et al., 2004; Redlich et al., 2007). Because of the more limited commercial use of TDI prepolymers and lack of sufficient toxicity data, the TDI RELs in this document apply only for exposure to TDI monomers (i.e., 2,4- and 2,6-TDI). See Section 7, Toxicity Studies of Toluene Diisocyanate Prepolymers, for summaries of TDI-prepolymer toxicity studies found in the open literature.

To minimize potentially harmful inhalation exposures, TDI has been replaced in many applications, especially consumer products, by other less volatile compounds such as methylene diphenyl diisocyanate (MDI), polymeric MDI (PMDI), and hexamethylene diisocyanate (HDI) prepolymers, so direct handling of TDI containing materials by consumers is less frequent. TDI and PMDI are the most commonly used diisocyanates for the manufacture of polyurethanes. They account for about 90% of world production involving diisocyanates (Redlich et al., 2007). HDI and its prepolymers and polyisocyanates are other commercially important isocyanates used principally as a hardener in spray paints and is listed on the California Toxic Air Contaminant list (Fent et al., 2008; CARB, 2010). Other diisocyanates available include naphthylene diisocyanate (NDI), isophorone diisocyanate (IPDI) and dicyclohexylmethane diisocyanate or hydrogenated MDI (HMDI) but their use is limited to more specialized applications. All of these diisocyanates are also known to cause asthma in occupational settings.

4. Metabolism

TDI is characterized by the N=C=O group which contains two double bonds and exhibits strong chemical reactivity (Raulf-Heimsoth and Baur, 1998). Animal inhalation studies with ^{14}C-labeled TDI showed that ^{14}C is found in the epithelium and the subepithelial level from the nose down to the terminal bronchioles, but is mainly absorbed in the upper airways (Kennedy et al., 1989). The uptake of ^{14}C-label into the blood is linear during exposure at concentrations ranging from 0.05 to 146 ppb.

Based on experiments in rats exposed to 2,4-TDI by inhalation, oral or iv routes, the metabolic scheme in Figure 2 was proposed by Timchalk et al. (1994). As with other isocyanates, TDI can readily react with hydroxyl, sulfhydryl and amine groups on macromolecules found in airway epithelial cells, serum and skin, including hemoglobin, glutathione, laminin, albumin, keratin and tubulin (Brown and Burkert, 2002; Bello et al., 2004). In the gut, hydrolysis of TDI generates toluene diamine (TDA), a carcinogen. Free TDA may be absorbed and be further metabolized, or may react with TDI to form polyurea polymers that are poorly absorbed and thus eliminated in the feces.

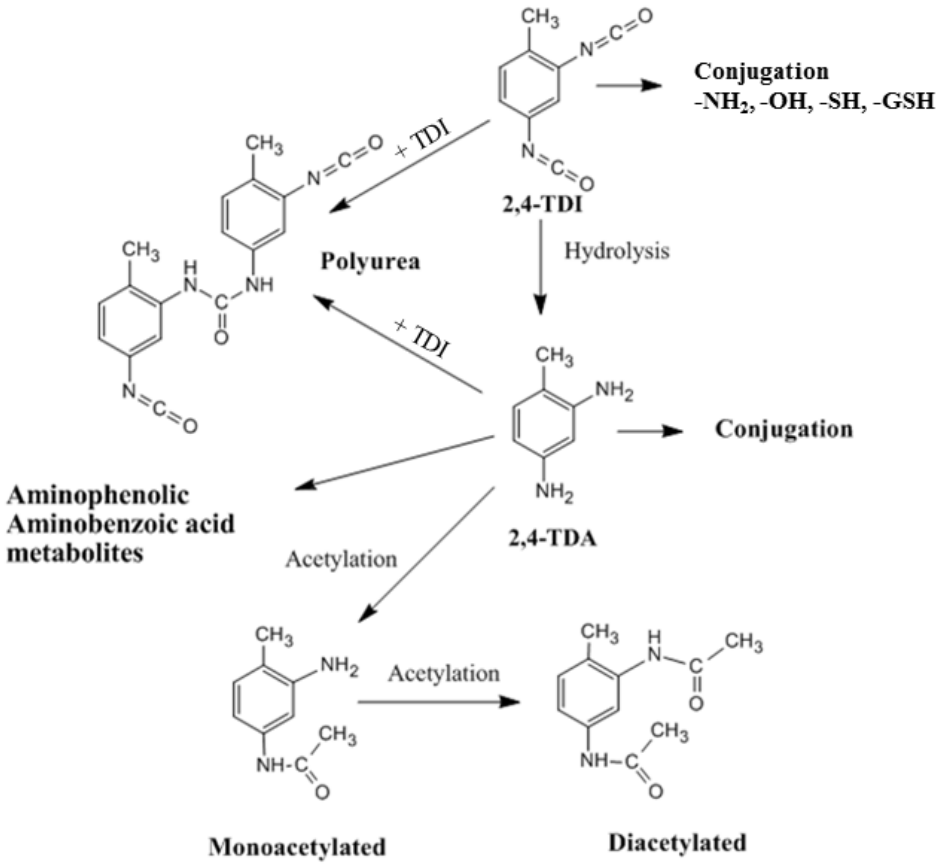


Figure 2. Modified Metabolic Scheme for TDI in Rat from Timchalk et al. (1994)

In experimental administration by the oral route, 12-20% of the dose was absorbed, while by the inhalation route, essentially all the TDI was absorbed (Timchalk et al., 1994). At 48 hours post-inhalation exposure, approximately 15% and 47% of the recovered metabolites were in urine and feces, respectively. The half-life for urinary excretion of metabolites following inhalation of TDI by the rats was 20 hrs. Inhalation exposure leads preferentially to the formation of TDI conjugates and little or no measurable TDA (Timchalk et al., 1994; Lindberg et al., 2011). These route-dependent differences in fate are posited to explain the observed carcinogenicity of TDI by the oral (with conversion to TDA) but not the inhalation route in experimental animals (Collins, 2002).

Kennedy et al. (1994) reported that the majority (74-87%) of the ^{14}C-labelled TDI associated with the blood was recovered in the plasma, and of this, 97-100% of the ^{14}C existed in the form of acid-labile biomolecular conjugates. The majority of these conjugates were greater than 10-kDa, with the predominant high molecular weight conjugate being a covalently modified 70-kDa plasma protein. Since albumin has about the same molecular weight, the authors hypothesized that this was a TDI-albumin conjugate.

Skarping et al. (1991) exposed five human subjects to approximately 40 µg/m^3 (5.6 ppb) TDI for 7.5 hours and followed its elimination from urine and plasma over 28 hrs post-exposure. Urinary elimination of TDI (measured as TDA following hydrolysis of urine samples) followed a possible biphasic pattern with a rapid phase $t_{1/2}$ of 1.9 hrs for 2,4-TDA and 1.6 hrs for 2,6-TDA. The limited data for TDI in plasma suggest that a fraction of hydrolysable 2,4 and 2,6-TDA was present in plasma and increased during exposure. A portion of the plasma fraction was rapidly eliminated and another fraction remained in the plasma for an extended interval (>28 hrs).

Exposure to TDI also occurs via dermal absorption and can lead to both dermal and pulmonary hypersensitivity (Karol et al., 1981). The metabolic fate of TDI following dermal exposure is expected to be similar to that following inhalation.

In occupational studies, a correlation between airborne exposure to TDI and urinary TDI metabolite concentrations has been found in workers (Geens et al., 2012). Urinary samples were hydrolyzed with sodium hydroxide to release the TDI-related diamines 2,4- and 2,6-TDA and then quantified as total TDA. Through regression analysis, a post-shift minus pre-shift TDA urine concentration of 18.12 µg/L corresponded to an airborne TDI concentration of 5 ppb (37 µg/m^3). A combined half-life of TDA in urine was 1.1 days, indicating that TDI metabolites may accumulate in the body of workers during the workweek.

Although TDI and other diisocyanates are a leading cause of chemically-induced occupational asthma, the mechanisms of disease pathogenesis have only recently begun to be understood. Research in this area has been conducted mostly with MDI and is also described here due to its similar metabolic pathway with TDI. TDI and MDI have been shown to react with GSH in lung lining fluid

and can then be absorbed into the bloodstream (Wisnewski et al., 2011; Wisnewski et al., 2013). *In vitro* studies have shown that GSH can act as a "shuttle" for TDI and MDI, in that once GSH-TDI and GSH-MDI is absorbed, TDI- and MDI-albumin conjugates are generated via GSH-mediated transcarbamoylation, which exhibit distinct changes in conformation and charge. MDI-albumin conjugates were specifically recognized by serum IgG of MDI workers with diisocyanate-induced asthma, suggesting one possible pathway for MDI in promoting immune responses (Wisnewski et al., 2013).

In another study, hybridomas secreting anti-MDI monoclonal antibodies were derived from mice immunized with self (serum)-proteins, which had been conjugated with MDI *ex vivo* (Wisnewski and Liu, 2013). Molecular characterization of the hybridomas' rearranged cDNA identified clonally distinct antibody heavy and light chain combinations that encode MDI recognition. The secreting clones were identified in initial screening ELISAs, based on differential binding to MDI conjugated human albumin vs. mock exposed albumin. The monoclonal antibodies secreted by the hybridomas also recognized MDI conjugated to other model proteins (e.g., ovalbumin, transferrin), but did not bind unconjugated proteins, or protein conjugates prepared with TDI or HDI. These data provide insight into the molecular determinants of humoral MDI specificity, and characterize anti-MDI IgG1 monoclonal antibodies that may be developed into useful diagnostic reagents.

In mice immunologically sensitized to MDI via prior skin exposure, GSH-MDI reaction products delivered intra-nasally induced significantly greater airway eosinophilia and mucus production, both hallmarks of asthma, than in naïve mice without prior MDI skin exposure (Wisnewski et al., 2015). Local airway inflammatory responses to GSH-MDI were characterized by markers of alternative macrophage activation and selective increases in the shared beta subunit of IL-12/IL-23 but not the respective alpha subunits or other asthma-associated Th2-type cytokines. The IL-12/IL-23β subunit is produced largely by macrophages/dendritic cells and, to a lesser extent, B-cells. These findings describe a GSH-mediated pathway that may distinguish the pathogenesis of isocyanate asthma from that triggered by other allergens.

Kim et al. (2010) observed that the expression of ferritin light chain (FTL) was decreased in both BALF and serum of workers (n=74) with TDI-induced asthma compared to asymptomatic exposed controls (n=144) and nonexposed controls (n=92). Ferritin is an iron storage protein consisting of two subunits, a heavy chain and light chain that sequester iron in the ferric (Fe^{3+}) state. Ferritin expression is regulated by oxidative stress via modifications of iron regulatory protein activity. The ability of cells to induce rapid ferritin synthesis prevents the effects of free radical damage to cellular components. Alternatively, transferrin was increased in serum of workers with TDI-induced asthma compared to asymptomatic exposed controls and nonexposed controls. Hypotransferrinemia is associated with resistance to oxidant injury.

Culture of A549 cells, a human epithelial cell line, with TDI resulted in a down regulation of FTL in a time- and dose-dependent manner (Kim et al., 2010). Although these were *in vitro* studies, they suggest that TDI may down regulate FTL expression in airway epithelial cells directly. Kim and colleagues also investigated the effects of TDI on heme oxygenase-1 HO-1), which catalyzes the degradation of heme, a potent oxidant. HO-1 activity is linked to FTL expression, in that ferritin is regulated in part by intracellular iron levels at both transcriptional and translational levels. TDI was also found to down-regulate HO-1 expression in A549 cells in a time- and dose-dependent manner. TDI also down-regulated the mRNA and protein levels of several anti-oxidant proteins such as thioredoxin-1, glutathione peroxidase-1, peroxiredoxin 1 and catalase as well as FTL and HO-1.

Finally, Kim et al. (2010) investigated the transcription factor Nrf2. The expression of several anti-oxidant proteins is regulated by Nrf2 by binding the anti-oxidant response element (ARE) in the promoter of the target genes. TDI did not change the total level of Nrf2. However, it did suppress nuclear translocation of Nrf2 through suppression of phosphorylation of mitogen-activated protein kinases, therefore, suppressing the binding of Nrf2 to the ARE region of the HO-1 promoter. Thus, the authors concluded that TDI inhibited FTL/HO-1 expression in A549 cells directly by regulating the mitogen-activated protein kinase-Nrf2 signaling pathway, which, if reproduced *in vivo*, may contribute to the development of airway inflammation in TDI-induced asthma.

The *in vitro* reaction of TDI with calf thymus DNA resulted in adduct formation (Jeong et al., 1998). The reactive form could be either TDI itself or may derive from the metabolic activation of the aromatic diamine derivative formed by hydrolysis (Bolognesi et al., 2001).

5. Acute Toxicity of Toluene Diisocyanate

The main effect of acute exposure to TDI in previously non-exposed individuals is sensory irritation. In experimental animals, this has been measured as respiratory rate depression that results from stimulation of trigeminal nerve endings located in the nasal mucosa. At a high enough dose, TDI can overcome the scrubbing ability of peptides and proteins in fluid of the upper airways and subsequently reach susceptible lung structures in the posterior airways. Thus, TDI also acts as a pulmonary irritant that is measured as a depression in tidal volume resulting from stimulation of the vagus nerve (Castranova et al., 2002; Pauluhn, 2014). Pulmonary irritation is also associated with an influx of neutrophils into bronchoalveolar airspaces. TDI prepolymers, on the other hand, are primarily aerosols in air that behave as pulmonary irritants rather than sensory irritants (Pauluhn, 2004).

If acute exposure is severe enough, an asthma-like condition known as reactive airways dysfunction syndrome (RADS) may occur that can persist for years. Subsequent exposure to low-level TDI or other irritants in these individuals

results in pulmonary symptoms including bronchial hyperresponsiveness and airflow obstruction.

Repeated inhalation exposure usually on the order of months to years may result in allergic sensitization. Sensitization generally refers to induction of heightened airways responsiveness that results in the development of a hyperactive state characterized by abnormal respiratory responses, such as asthmatic symptoms. However, the minimum TDI exposure that can lead to sensitization or asthma remains unclear (Redlich et al., 2007). In addition, the role of various exposure factors, such as peak vs. cumulative exposure, and route of exposure (e.g., respiratory, skin), are not well defined. In animal models, significant dermal exposure to TDI or MDI leading to systemic sensitization may not only lower the subsequent inhalation dose necessary to produce an asthma-like response, but also result in a more severe inflammatory response (Pauluhn and Poole, 2011; Pauluhn, 2014; Wisnewski et al., 2015).

Asthmatic cross reactivity between different isocyanates has been documented. Innocenti et al. (1988) found that nearly 50% of subjects with asthma induced by TDI also exhibited asthmatic reactions to MDI, which they were never exposed to at work. In another study, of 13 workers exclusively exposed to MDI, four also reacted to TDI (O'Brien et al., 1979a). In six workers with IgE-mediated sensitization to isocyanates, radioallergosorbent test (RAST) and/or skin-test investigations revealed the presence of IgE antibodies reacting specifically with isocyanates conjugated with human serum albumin (HSA); these isocyanates included those to which workers were exposed as well as other isocyanates to which they had not been exposed (Baur, 1983). These results indicate the predominance of closely related antigenic determinants in HSA conjugated with different isocyanates. The common antibody-binding regions are recognized to different extents by antibodies of clinically sensitized workers, indicating individual differences in specificities and avidities of antibody populations.

5.1. Acute Toxicity to Adult Humans

Acute respiratory exposures to TDI are typically reported in occupational settings with responses ranging from upper airway irritation to toxic bronchitis (Ott et al., 2003). Eye, nose and throat irritation are often the first manifestations of acute high exposure to TDI, with dry cough, and chest pain and tightness ensuing. Patchy infiltrates may be seen on chest X-rays, and the clinical picture may approximate bronchitis, bronchiolitis, bronchial asthma, or pneumonitis (Peters and Wegman, 1975). Once sensitized to TDI, a subsequent acute inhalation exposure, often considerably below the odor and sensory irritation threshold (i.e., <5-20 ppb), may provoke a potentially life-threatening pulmonary hypersensitivity response.

The American Conference of Governmental Industrial Hygienists (ACGIH) has a 5 ppb threshold limit value (TLV, 8 hr time weighted average) and 20 ppb short term exposure limit value (15 min time weighted average) for worker exposure.

These concentrations represent levels at which irritant effects on the mucosa are unlikely to occur. They are not meant to represent levels at which sensitization is unlikely to occur, nor do they represent levels that are protective for workers already sensitized. In 2006, the ACGIH proposed lowering of the TLV to 1 and 3 ppb for 8 hr and 15 min exposure, respectively (Geens et al., 2012). Since 2009, the proposed TDI TLV has been on the ACGIH "notice of intended change" list. The U.S. Occupational Safety and Health Administration (OSHA) has a Permissible Exposure Limit ceiling of 20 ppb, a level that may not be exceeded for any period of time (U. S. OSHA, 2015). Ceiling limits are applied to irritants and other materials that have immediate effects. U.S OSHA does not have an 8-hour TWA exposure limit, whereas California OSHA has an 8-hr TWA exposure limit of 5 ppb.

5.1.1. Dose-Response Chamber Studies in Humans

Henschler et al. (1962) conducted one of the only controlled human exposure studies with exposure concentrations of TDI considerably above 20 ppb. In the German article (translated into English by OEHHA), six healthy men were exposed to various concentrations of TDI 65/35 (2,4-TDI and 2,6-TDI, respectively) and to the pure isomers for 30 min in an exposure chamber. The subjects were only exposed to one concentration of TDI per day, which was randomly selected. At 0.01 and 0.02 ppm (10 and 20 ppb) neither odor nor other sensory symptoms were perceived. At 0.05 ppm (50 ppb), the odor of TDI was perceived immediately upon entering the chamber, but was not noticeable by the men after 4-9 min of exposure. Three of 6 subjects noted conjunctiva irritation by 15 min of exposure, which was described as "tear stimulus, but without tearing".

At 0.075 ppm TDI 65/35, the odor was stronger and took longer to become unnoticeable (12-14 min). A light burning sensation of the eye without tearing was experienced in the subjects after 1-6 min of exposure. When asked to breathe deeply, all felt a tingling or slight stinging sensation in the nose. Exposure to 0.1 ppm produced more severe eye and nose irritation described as resembling a cold. Odor disappeared after 12-15 minutes.

At 0.5 ppm, lacrimation was elicited in all subjects, but the eye irritation was still considered bearable. A strong stinging sensation and greater secretion from the nose were noted. The throat was described as scratchy or burning, but without coughing. Two subjects were exposed to 1.3 ppm TDI 65/35 for 10 min resulting in heavy eye tearing, eye reddening and eyelid closure. Several hours after exposure, significant catarrhal symptoms (mucous membrane inflammation) of the respiratory tract appeared with coughing.

Henschler et al. (1962) also exposed subjects to pure 2,4-TDI and 2,6-TDI individually. Exposure to 0.02 ppm 2,4-TDI did not result in sensory symptoms, but a weak odor was noted at 0.05 ppm. No eye irritation was noted. Irritation of the conjunctiva and nose occurred at 0.08 ppm, and was significantly stronger with exposure to 0.1 ppm. Eye irritation occurred in 2 out of 5 subjects at 0.2

ppm, while 0.5 ppm caused tearing, which was described as piercing and annoying in all subjects. Exposure to 2,6-TDI was described as having similar sensory effects as that produced by 65/35 TDI, but was considered slightly stronger than the same concentration of 2,4-TDI. At 0.5 ppm 2,6-TDI, the subjects could not differentiate from the effects produced by 2,4-TDI at the same concentration.

The threshold for odor recognition above 20 ppb reported by Henschler et al. (1962) is supported by numerous reports of exposures of 5-20 ppb resulting in no noticeable odor. Other acute studies that were primarily conducted to assess or confirm TDI-sensitization in workers also found no sensory irritation in normal humans with exposures up to 20 ppb for 30 min or less. In support of the finding of sensory irritation at 50 ppb and above by Henschler et al. is a report by Lee and Phoon (1992) in which uncontrolled industrial exposure to a mean concentration of 160 ppb TDI (range: 10 to 500 ppb) resulted in eye and respiratory system irritation. Similarly, painters handling polyurethane varnish experienced eye, nose and throat irritation with exposure to TDI concentrations of 70-170 ppb (Huang et al., 1991).

5.1.2. Challenge Studies in Sensitized Workers

There are numerous reports in the literature in which low acute concentrations of TDI were used to study or confirm a diagnosis of probable diisocyanate asthma in workers (Chester et al., 1979; Mapp et al., 1986; Moller et al., 1986a; Boschetto et al., 1987; Banks et al., 1989; Vogelmeier et al., 1991; Karol et al., 1994; Pisati et al., 2007). Specific inhalation challenge has been referred to as the “gold standard” for diagnosis, since clinical history, questionnaires and physiological studies are frequently not definitive by themselves. Concentrations of TDI in these challenge tests usually ranged between 5 and 20 ppb with exposure durations of 10 min to several hours. With a few exceptions, concentrations of TDI higher than 20 ppb are not used in controlled human studies due to the potential to provoke acute allergic sensitization, sensory irritation and respiratory inflammation.

In studies of workers suspected to have TDI-induced asthma, pulmonary responses often resulted from exposures below 10 ppb TDI. The lowest controlled exposure to TDI resulting in an asthmatic response is ≤1 ppb. Lemiere et al. (2002) exposed eight subjects with occupational asthma induced by specific diisocyanates (TDI, MDI or HDI) to 1 ppb using a closed circuit apparatus. The authors considered a positive result to be a 20% or greater reduction in Forced Expiratory Volume in 1 second (FEV_1). By this criterion asthma symptoms were triggered in two of the subjects with a 30 min exposure, one to MDI and the other to HDI. A third subject had asthma symptoms with a 45 min exposure to TDI. There was also a significant correlation (Spearman rank order test $\rho=0.8$, $P<0.001$) between the percentage of maximum decrease in FEV_1 after exposure to 1 ppb and the increase in sputum neutrophil count, indicating inflammatory changes as well.

In another study of workers suspected to have TDI-induced asthma, 9 out of 63 subjects responded with a fall in FEV_1 of 15% or more following exposure to a TDI concentration of ≤1 ppb for 30 min (O'Brien et al., 1979b). This sensitive subgroup of TDI responders also showed a significantly greater increase in bronchial reactivity to both histamine and exercise than the less sensitive subgroup with asthmatic reactions to TDI concentrations of 2-20 ppm (28 subjects), and the TDI non-sensitive group (26 subjects).

5.1.3. Challenge Studies in Normals and Non-Sensitized Asthmatics

More pertinent to derivation of an acute REL for TDI, some studies also tested the pulmonary response in exposed normal and asthmatic subjects with no sensitization or history of exposure to isocyanates, in addition to testing the pulmonary response in workers with probable TDI sensitization.

Vogelmeier et al., 1991; and Baur and Colleagues, 1994

Diisocyanate inhalation tests were performed in exposure chambers on 19 workers with diisocyanate-induced asthma and on 10 healthy and 15 asthmatic volunteers with no previous contact with diisocyanates (Vogelmeier et al., 1991; Baur et al., 1994). The ten healthy individuals had a negative methacholine test and were exposed to 20 ppb TDI for 2 hrs. The 15 patients with asthma had a positive methacholine test and were exposed for one hr to 10 ppb TDI, followed by a 45 min break, then a one hr exposure to 20 ppb. Pulmonary function tests included airway resistance (Raw), specific airway conductance, FEV_1, inspiratory vital capacity, and total lung capacity. The pulmonary function test results of volunteers without previous contact with diisocyanates were presented by two different approaches: Vogelmeier et al. (1991) used a $\geq$50% decrease in specific airway conductance from the zero value as evidence of a positive airway reaction, while Baur et al. (1994) used a 100% increase of airway resistance as evidence of a positive airway reaction.

In the approach used by Vogelmeier et al. (1991), one of 10 normal volunteers showed a positive airway reaction ($\geq$50% decrease in specific airway conductance) to 20 ppb TDI, and one of 15 subjects with asthma had a positive airway reaction to 10 ppb TDI. With subsequent exposure of the asthmatic subjects to 20 ppb for 1 hour, two of the remaining 13 asthmatic subjects had a positive reaction to 20 ppb TDI. (Baur et al. (1994) later clarified that the subject responding to 10 ppb of TDI and another subject with asthma who refused to continue did not undergo the second challenge with 20 ppb TDI.) All the positive airway reactions occurred during the first hour after inhalation; late responses were not observed. Vogelmeier et al. concluded that supposedly sub-irritant concentrations of TDI may induce a marked airway reaction in healthy volunteers and patients with asthma.

Baur et al. (1994) then presented the Raw results measured by body plethysmography on the same subjects. Using 100% increase in Raw

(amounting to values of ≥0.5 kPa/l per second) as evidence of a positive airway reaction, none of the 10 healthy controls had an asthmatic response with a two-hour exposure to 20 ppb TDI. In contrast, one of 15 asthmatics responded to 10 ppb with a severe asthmatic reaction. Another asthmatic subject from the remaining group of 13 subjects showed an asthmatic reaction to 20 ppb. Prior to challenge testing, the asthmatic subjects were categorized based on their bronchial reactivity to acetylcholine (Ach). Three of these subjects responded to <0.1 mg Ach with an increase in Raw of 100% (PD_{100}), the lowest Ach level of the group. Two of these asthmatic subjects were also the ones that responded to TDI challenge at 10 and 20 ppb with an increase in airway resistance of >100%. The other asthmatic subjects had a PD_{100} for Raw of 0.1-0.4 mg Ach (5 subjects) and 0.4-1 mg Ach (7 subjects). None of the 10 healthy controls responded to 1 mg Ach with an increase in Raw of 100%.

Based on these results, Baur et al. presented a different conclusion from Vogelmeier et al. (1991). Baur et al. concluded that the TDI exposures in the TLV range (5 to 20 ppb) do not change lung function in healthy subjects and only rarely in previously unexposed asthmatics, indicating that asthmatic responses in TDI challenge tests are not absolutely specific to sensitized individuals.

Additional details of this study are in Baur (1985), but were unavailable to OEHHA. However, this study was summarized by NRC (2004). Of the 15 asthmatic subjects exposed to TDI, five complained of chest tightness, rhinitis, cough, dyspnea, throat irritation, and/or headache during exposure. It was unclear from the report what concentration of TDI caused these symptoms. Three normal subjects reported eye irritation and/or cough. Among the asthmatics, no decrease in FEV_1 >20% was observed, although two showed a decrease in FEV_1 between 15 and 20%. Increases in Raw did not correspond with decreases in FEV_1, and neither parameter could be used as an indication of the reported symptom discomfort.

Fruhmann et al., 1987

A group of subjects was examined using similar methodology, as conducted by Baur et al. (1994) and Vogelmeier et al. (1991) and involved many of the same researchers. In this German study by Fruhmann et al. (1987), translated into English by OEHHA, 15 healthy and 15 asthmatic subjects with no previous contact with diisocyanates were exposed to TDI, and airway resistance (Raw) was recorded periodically by whole body plethysmography. Raw was measured in kilopascals per liter per second ($kPa.s.L^{-1}$). The healthy subjects were chamber-exposed to 20 ppb TDI for 2 hrs, while the asthmatic subjects were exposed to 10 ppb TDI for 1 hr, then given a break for 45 min followed by a 1 hr exposure to 20 ppb TDI. For healthy subjects, mean Raw before and after exposure was 0.12 and 0.17 $kPa.s.L^{-1}$, respectively, with no individual experiencing an increase in Raw above 0.25 $kPa.s.L^{-1}$. A normal Raw result was considered to be <0.35 $kPa.s.L^{-1}$. For the asthmatic subjects, significant changes and complaints were recorded in one-third of the subjects, although the authors

did not specify what these changes and complaints were other than presenting the individual Raw results. Three of the 15 asthmatic subjects experienced a maximum Raw value >100% of their control value taken before exposure. Raw was recorded during and up to 3 hrs post-exposure, but it was not specified exactly when the highest Raw value was recorded. Another five asthmatic subjects had a maximum increased Raw between 50-100% of their control value, all of which were above 0.35 kPa.s.L^{-1}.

Chester et al., 1979

Chester et al. (1979) exposed 40 subjects by facemask to 20 ppb TDI for 20 min and then assessed airway response by specific airway resistance (SRaw) at regular intervals for up to six hours following exposure. Twenty of the subjects were symptomatic TDI workers and the other subjects were a nonsmoking control group consisting of 20 subjects (10 healthy subjects and 10 subjects with extrinsic (allergic) asthma) without previous TDI exposure. The authors defined symptomatic TDI workers as those that have experienced bronchoconstriction with occupational TDI exposure. Using an increase in SRaw$\geq$50% above baseline resistance as a positive response to the TDI challenge, Chester et al. found that 9 of the 20 TDI workers were positive responders (one immediate, five dual and three late asthmatic reactions). None of the extrinsic asthmatics or normal subjects responded to 20 ppb TDI by an increase in their SRaw greater than 50%. The specific changes in SRaw experienced by the asthmatics and normals were not presented by the authors.

Some control subjects and seven of the non-responding TDI workers were then assessed for small airway function by testing for maximum expiratory flow volume by breathing air and repeated when breathing helium-oxygen (Chester et al., 1979). Using the criteria of a 40% increase in the volume of isoflow and a 40% decrease in Forced Expiratory Flow at 50%FVC (ΔFEF_{50}) as evidence of small airway changes, the authors observed that five of seven “non-responders” had reduced lung function. Of the normal subjects and seven subjects with extrinsic asthma examined using these pulmonary function tests, none were considered responders by the criteria applied.

Fabbri et al., 1987

Fabbri et al. (1987) exposed 6 normal subjects with no previously documented asthmatic reaction to 18 ppb TDI for 30 min. FEV_1 and airway responsiveness to methacholine were unaffected by TDI exposure.

Moller et al., 1986

Moller et al. (1986b) exposed 10 subjects, with a positive methacholine challenge test and no apparent previous exposure to TDI, to concentrations of TDI up to 20 ppb for 15 min. A positive methacholine test was considered to be a fall in FEV_1 of 20% or greater when exposed to a total cumulative dose of <2,000 µg

methacholine. No change in FEV_1 was observed with exposure to TDI in these subjects.

Mapp et al., 1986

In addition to exposing a group of 40 sensitized TDI-workers (10 each with immediate, dual, late, or no asthmatic reactions after exposure to TDI) to 18 ppb TDI for up to 30 min, Mapp et al. (1986) also exposed eight asthmatic subjects with no history of sensitization to TDI. FEV_1 was measured immediately before and after exposure and then hourly for 8 hrs. The provocative dose of methacholine that had previously caused a decrease in FEV_1 of 20% (PD_{20}, the dose of methacholine in mg) was also measured. An asthmatic reaction was considered to occur when FEV_1 decreased by 20% from baseline, or an increase in airway responsiveness occurred when the PD_{20} FEV_1 decreased at least two-fold. By this measure, exposure to TDI did not elicit an asthmatic reaction in the group of asthmatic subjects not sensitized to TDI. Although airway responsiveness was markedly increased with methacholine challenge in asthmatic subjects not sensitized to diisocyanates, the authors found no further decrease in FEV_1 after TDI inhalation with challenge at the PD_{20}.

Vandenplas et al., 1999

Vandenplas et al. (1999) exposed 17 subjects without respiratory symptoms (eight smokers and nine nonsmokers) or occupational exposure to diisocyanates to ambient air and once to 5 ppb TDI for 6 hrs followed by 20 ppb TDI for 20 min. Nonspecific bronchial responsiveness was assessed by inhalation of histamine, which was expressed as the concentration of histamine causing a 20% fall in FEV_1. Several pulmonary function tests including specific airway conductance (sGaw), functional residual capacity (FRC), total lung capacity (TLC), forced vital capacity (FVC), FEV_1, FEV_1/FVC ratio, and maximal expiratory flow at 50% of FVC ($MEF_{50\%}$), and at 25% of FVC ($MEF_{25\%}$), were carried out before exposure and at every hour during exposure. Bronchial lavage (BL) and bronchoalveolar lavage (BAL) were performed 1 hr after each exposure.

None of the subjects in the Vandenplas et al. (1999) study experienced significant respiratory symptoms in response to the exposures. Comparison of pre- and post-exposure pulmonary function values did not result in significant differences. The pulmonary function tests did find detectable changes in airway caliber throughout the exposure period using regression analysis of repeated measures. Compared to ambient air exposure, TDI exposure resulted in a modest decrease in sGaw ($p=0.053$) and in $MEF_{25\%}$ ($p=0.015$). Multivariate analysis of the time-point differences in sGaw showed that the mean concentration of TDI was a significant determinant of the response, while the level of nonspecific responsiveness to histamine had a significant effect on changes to $MEF_{25\%}$ induced by TDI exposure. The authors suggest these results show that TDI could exert an effect on both small and large airways.

TDI exposure in the Vandenplas et al. (1999) study also resulted in a slight increase in BAL albumin level (TDI: 26.4±12.5 µg/ml vs. air: 21.8±8.6 µg/ml, p=0.044) and in BL α_2-macroglobulin concentration (TDI: 0.07±0.061 µg/ml vs. air: 0.05±0.04 µg/ml, p=0.044). The authors note that the observed increase in BAL albumin content after TDI exposure likely represents indirect evidence of changes in permeability of the epithelial barrier and slight leakage of blood plasma components into the alveolar compartment. The increase in BL α_2-macroglobulin level could reflect a selective increase in epithelial permeability associated with local production. The concentrations of potential indicators of epithelial cell dysfunction (secretory component and CC16) and pro-inflammatory cytokines (TNF- α, IL-4, IL-5, IL-6 and IL-8) were not significantly altered by TDI exposure, suggesting to the authors that the observed TDI-related changes in pulmonary function tests were not directly related to airway inflammation.

Raulf-Heimsoth et al., 2013

A standardized 4-step-in-1-day diisocyanate exposure approach was used on 63 diisocyanate-exposed workers with work-related symptoms and 10 controls with bronchial hyperresponsiveness, but without prior occupational exposure to diisocyanates (Raulf-Heimsoth et al., 2013). The subjects underwent challenge testing with the dominant diisocyanate used at their work: TDI in 6 cases, MDI in 40 cases, HDI in 18 cases and NDI in 2 cases. The reference group was challenged to both TDI and MDI. The exposure regimen was 5 ppb for 30 min, 10 ppb for 30 min, 90 min break, 20 ppb for 30 min, 90 min break, and 30 ppb for 30 min. The total diisocyanate exposure time was 2 hours.

Twelve out of 63 subjects suspected to have diisocyanate-induced asthma showed an FEV_1 decrease >20% after the challenge. Among the six potentially sensitized workers exposed to TDI at their work, two responded with a positive reaction. No similar respiratory response was reported by the authors in the non-exposed hyperresponsive reference group. Cellular composition and soluble inflammatory biomarkers were examined in nasal lavage and induced sputum samples. Nasal lavage was used to assess upper respiratory inflammation, while induced sputum was used to assess lower airway inflammation. The major finding was increased eosinophils, eosinophil granule-derived cationic protein and IL-5 in induced sputum of workers responding with an FEV_1 decrease >20% with diisocyanate challenge. Increases in these biomarkers were not observed in non-responding workers and the hyperresponsive, non-exposed reference group. No significant differences in cellular composition and soluble inflammatory markers were found in nasal lavage fluid of any of the three groups. The authors concluded that an influx of eosinophils measured in induced sputum was the primary indicator of lower airway inflammation in workers that respond to diisocyanate challenge, and that the upper airways are not significantly affected by diisocyanates at these doses.

A summary of the acute studies presented above is shown in Table 2.

Table 2. Summary of Controlled Acute TDI Exposure Studies in Naïve Subjects

Study	TDI Exposure Conditions	Pulmonary/Sensory Findings
Henschler et al. (1962)	6 subjects, 1 exposure/day 30 min exposure to: 10, 20. 50, 75, 100, 500, and 10 min exposure to 1300 ppb	No symptoms at 10 or 20 ppb; increasing sensory irritation with increasing TDI concentration starting at 50 ppb and above.
Vogelmeier et al., 1991; Baur et al., 1994	10 normal subjects, 20 ppb for 2 hrs 15 asthmatics, 10 ppb for 1 hr, 45 min break, then 20 ppb for 1 hr	Normals: No significant pulmonary decrement; 3 complained of eye irritation and/or cough Asthmatics: 1/15 had ≥100% increase in Raw at 10 ppb; 1/13 had ≥100% increase in Raw at 20 ppb; overall, 5 complained of chest tightness, rhinitis, cough, dyspnea, throat irritation, and/or headache
Fruhmann et al., 1987	15 normal subjects, 20 ppb for 2 hrs 15 asthmatics, 10 ppb for 1 hr, 45 min break, then 20 ppb for 1 hr	Normals: No significant increase in Raw Asthmatics: 3/15 had ≥100% increase in Raw; one-third of subjects experienced significant, but unspecified, changes or complaints
Chester et al. (1979)	10 normal subjects and 10 asthmatics 20 ppb for 20 min	No increase in SRaw greater than 50% in any subject
Fabbri et al. (1987)	6 normal subjects 18 ppb for 30 min	No change in FEV_1 or airway responsiveness to methacholine
Moller et al., 1986	10 subjects with positive methacholine challenge test, up to 20 ppb for 15 min	No change in FEV_1 observed with methacholine challenge after TDI exposure
Mapp et al., 1986	8 asthmatic subjects 18 ppb for 30 min	No decrease in FEV_1 ≥20% observed; No decrease in the PD20 FEV_1 greater than 2-fold with methacholine challenge
Vandenplas et al. (1999)	17 normal subjects 5 ppb for 6 hrs followed by 20 ppb for 20 min, with pulmonary function test every hr	Decreased s*G*aw (p=0.053) and $MEF_{25\%}$ (p=0.015) measured by regression analysis of repeated measures; increased BAL albumin level (p=0.044) and BL macroglobulin (p=0.044) concentration
Raulf-Heimsoth et al. (2013)	10 non-exposed subjects with bronchial hyperresponsiveness 5, 10, 20 and 30 ppb for 30 min each	No FEV_1 decrease >20% observed; No increase in eosinophils and soluble inflammatory biomarkers in nasal lavage and induced sputum

5.2. Acute Toxicity to Infants and Children

No studies were located that examined the effects of acute exposure to TDI in children. However, the effects due to acute TDI exposure would be expected to be similar to those reported following acute accidental exposure of school children to the diisocyanate MDI (Jan et al., 2008). In this report, asthma-like symptoms were observed among 203 Taiwanese schoolchildren during a school track paving/spraying operation of an MDI mixture at 870 ppm w/w in xylene. The air concentration of the MDI and xylene that the children were exposed to is unknown. Acute symptoms were observed when the wind suddenly changed direction and blew the emissions towards nearby classrooms. Of the exposed children, 70.9% reported headache, 67.5% had persistent cough, 63.5% had dyspnea, and 62.6% nausea. Chest discomfort was reported by 23.6% of the students but chest X-rays were normal. Bronchodilators were administered to 15.8% who experienced wheezing and difficulty breathing. The authors observed an inverse linear relationship between the incidence of affected students in various classrooms and the distance from the site of MDI spillage ($r = -0.48$, $p < 0.05$) suggesting a dose-response.

During follow-up surveillance three days after the incident, the prevalence of residual symptoms was cough 30.0%, headache 19.7%, dyspnea 15.3%, sore throat 10.3%, and nausea 3.9% (Jan et al., 2008). A positive history of asthma among 10.8% of the students was strongly correlated with the incidence of dyspnea (OR 4.09; 95% CI 1.17-14.32) and an abnormal pulmonary function test (OR 3.84; 95% CI 1.09-13.5). However, none of the other symptoms during the episode was correlated with either asthma history or abnormal lung function tests. In addition, 60.8% of the children without a history of asthma also complained of dyspnea, and 16.2% required bronchodilators for symptomatic relief. Acute exposure to high levels of MDI was thus associated with reactive airway dysfunction (RADS) among previously unexposed individuals. A spot urine test did not reveal a positive reaction for MDA in acid-hydrolyzed urine samples. The authors attributed this finding as characteristic of a brief exposure to MDI. There was no discussion of effects seen in exposed adults, so it is unclear if children were more prone to the acute effects of MDI than adults. Also, no apparent follow-up was performed to determine if the children had been immunologically sensitized as a result of the high acute exposure.

Jan et al. (2008) assumed all the symptomology was due to MDI even though xylenes are also known to cause acute eye and respiratory symptoms. Controlled acute exposures of human adult volunteers to 460-690 ppm xylenes resulted in transient eye and throat irritation and dizziness (Carpenter et al., 1975). In children living in high traffic density regions, upper airway or asthma symptom episodes were found to be associated with combustion-related xylene exposure (Buchdahl et al., 2000; Delfino et al., 2003). However, similar associations were found with other VOCs, and combustion-related gases. A proportion of the eye and respiratory effects could have been caused by xylene exposure, since xylenes are more volatile than MDI, and the formulation applied

to the track was composed primarily of xylenes. However, TDI (and possibly MDI) is about 10,000 times more potent (50 ppb for TDI vs. 460 ppm for xylenes) in causing acute eye and throat irritation in humans compared to xylenes.

Krone and Klingner (2005) have postulated that a relationship exists between exposure to polyurethane products made from isocyanates and childhood asthma. Further discussion is presented in Section 6.2.

5.3. Acute Toxicity to Experimental Animals

The effects on the respiratory tract of a single 4-hour exposure of mice, rats, guinea pigs and rabbits to 2, 5 or 10 ppm TDI were reported by Duncan et al. (1962). Two hours following termination of the exposure to 2 ppm, focal coagulation necrosis and desquamation of the superficial epithelium lining the trachea and the major bronchi were observed. Occasionally bronchial airways were found containing acute inflammatory cells associated with sloughed epithelium. However, by one day following exposure, acute inflammatory exudates were observed in the majority of the major bronchi, along with further desquamation of necrotic epithelium. Fibrinous strands were observed in the lumina, and an infiltration of polymorphonuclear leukocytes extended through the edematous peribronchiolar connective tissue. Acute inflammatory cells also infiltrated the edematous perivascular spaces of the accompanying vessels. By day four, there were signs of clearing of the inflammatory response and evidence of regeneration of tracheal and bronchial epithelium. By day seven, control and exposed animals were not significantly different. While the effects of exposure to 2 ppm were largely transient, exposures at 5 and 10 ppm resulted in more severe effects that were not completely reversible. The LOAEL for these effects was 2 ppm, but a NOAEL was not observed.

The effects of single, and repeated 3-hour exposures to 2,4-TDI vapor on sensory irritation at concentrations ranging from 0.007 to 2 ppm were measured as respiratory rate depression and nasal histopathology changes in mice (Sangha and Alarie, 1979). With single exposures, time-response relationships showed the slow development of the respiratory response with exposure duration. Concentration-response relationships also showed that the level of the response was dependent upon both exposure concentration and duration. Repeated exposures at ≥0.023 ppm resulted in cumulative effects. Regardless of the TDI concentration used, respiratory rates decreased relatively rapidly during the first 10 minutes of exposure, followed by a more gradual decline. As shown in Table 3, depression of the respiratory rate by 50% (RD50) was achieved in 10 min at a concentration of 0.813 ppm, in 60 min at 0.386 ppm, but took 180 minutes at 0.199 ppm.

Table 3. Respiratory Depression (RD50) Dependence on Exposure Duration

Exposure Time (min)	10	30	60	120	180	240
RD50 (ppm)	0.813	0.498	0.386	0.249	0.199	0.199

Recovery of respiratory rate following cessation of exposure was similarly duration dependent, being rapid with short exposures and slow with long exposures. This is in contrast to other sensory irritants such as acrolein, the response to which is only concentration dependent and recovery is rapid regardless of the exposure duration (Kane and Alarie, 1977). The slow rate of recovery became more evident when 3-hour exposures of 0.023, 0.078, 0.301, 0.505, 0.82, and 1.18 ppm were repeated on successive days. On the first day of exposure, the drop in respiration rate was similar to that of the single exposures: rapid during the first 10 minutes, with a more gradual descent thereafter. However, on subsequent days, the pre-exposure rates were progressively lower indicating incomplete recovery from the exposures on previous days. Recovery to baseline required at least five days after the last exposure. By contrast, with exposures in the range of 0.007 to 0.018 ppm, there were no consistent respiratory responses after the three-hour exposures, nor was there the pattern of reduced respiratory rate with repeated exposure. Histopathological evaluation of successive transverse sections of the nasal area revealed no lesions following three days of exposure for 3 hours/day to 0.031 ppm. However, in mice similarly exposed to 0.25 ppm, damage was consistently observed in the most anterior section of the nasal passages and external nares, with 25-50% of the mucosa involved including some extension into the submucosa. The lesions were much less pronounced in more distal sections.

These results suggest that, in addition to the potential for immune hypersensitization demonstrated in other studies (Karol et al., 1980), TDI has cumulative irritant effects that result from incomplete recovery from previous exposures above a certain level in this rodent model. Both the development of and recovery from these effects are slow, possibly consistent with covalent modification of receptors as in the reaction of TDI with OH or NH_2 groups in proteins and/or specific residues on TRPA channels in sensory neurons. TRPA belongs to the family of transient receptor potential (TRP) channels that transduce sensory neurons' response to thermal, mechanical, and specific chemical stimuli.

In guinea pigs, Gagnaire et al. (1988) demonstrated that TDI-induced airway hyperresponsiveness to intravenous acetylcholine can occur with continuous exposure as short as a single 4 hr exposure to 1.2 ppm. Similar responses to acetylcholine in guinea pigs were seen with TDI exposures to 118 ppb for 48 hr, 1.08 ppm for 4 hr/day for 2 days, and continuous exposure to 23 ppb for 1 week. However, regardless of exposure concentration or duration, TDI did not modify the baseline airway resistance (Raw). The authors concluded the study results were consistent with the hypothesis of a cumulative effect of TDI on airway hyperresponsiveness.

It is important to note that the development of pulmonary sensitivity to TDI does not require inhalation exposure. Working with guinea pigs, Karol et al. (1981) systemically sensitized guinea pigs to TDI by applying TDI in olive oil on the skin

at concentrations between 1 and 100%. Respiratory hypersensitivity could be demonstrated 14 days later by inhalation challenge to 5 ppb TDI or aerosols of TDI-protein conjugates. Pulmonary sensitivity was measured as increases in respiratory rates greater than three standard deviations (SD) from the mean upon subsequent inhalation challenge. Rapid, shallow breathing is an early response to chemical stimulation of bronchial C-fibers (Coleridge et al., 1983). Significant pulmonary sensitivity was seen in 2 of 12 animals challenged with 5 ppb TDI vapor, in 4 of 12 animals challenged with TDI-protein conjugates, in 5 of 12 animals challenged with toluene monoisocyanate-protein conjugates, and in none of the animals challenged with the unconjugated protein carriers alone.

Marek et al. (1995) investigated the induction of lung injury and development of airway hyperresponsiveness in rabbits with acute exposure to 5, 10 or 30 ppb TDI. Eight rabbits per group were anesthetized and intubated for pulmonary function tests and to increase the effective dose in the lower airways and the lung. Exposure to TDI four times each over a period of one hour did not significantly alter airway resistance, dynamic elastance, slope of the inspiratory pressure generation, arterial pressure or arterial blood gas tensions. Airway responsiveness to aerosols of 2% acetylcholine (Ach) was measured before and after each TDI exposure. No effect by Ach was seen in rabbits exposed to 5 ppb TDI for up to 7 hrs. With exposure to 10 ppb TDI, the amplitude of the Ach-constrictor response, indicated by changes in dynamic elastance, had increased by almost 2-fold by the third hour of TDI exposure, with similar increases in airway resistance and the slope of the inspiratory pressure generation also recorded. Exposure to 30 ppb TDI resulted in a steeper increase in these pulmonary responses following 2% Ach exposure. The authors concluded that the increased airway resistance to Ach in the rabbits exposed to TDI is an early indication for the development of obstructive lung disease.

Since TDI exposure may elicit both pulmonary and immune responses, it is of interest to compare the relative levels of exposure that elicit these respective responses. Guinea pigs received whole body exposure to 0, 0.02, 0.2, 0.6, or 1.0 ppm TDI as an aerosol comprising an 80:20 mixture of the 2,4- and 2,6-isomers 3 hr/day for 5 days (Aoyama et al., 1994). Three weeks following these induction exposures, all animals were challenged with a 15 min exposure to 0.02 ppm TDI while in a body plethysmograph to measure respiration rates. The pulmonary response was assessed as the percentage increase in respiratory rate. Compared to controls, an increase of at least 3 standard deviations measured during and 60 minutes after the challenge exposure was considered significant. By this criterion, animals induced by exposure to 0.2 ppm and above showed significant pulmonary responses. There was, however, no linear correlation between the intensity of the pulmonary responses and the dose used for induction. Whether this is related to the ability of TDI to act both as a sensory irritant, thereby decreasing respiratory rate, and as a pulmonary irritant that increases respiratory rate is not clear. Alternatively, the breathing rate during induction was not reported and may have decreased, consistent with TDI's sensory irritating properties.

The breathing rate increase measured during the subsequent challenge by Aoyama et al. (1994) may reflect pulmonary changes associated with the immune response. The number of animals responding at each dose level was also not different among exposure groups. The time course of IgG production was followed and the first TDI-specific antibodies were detected in some of the animals 6 days following the first induction exposures to 0.2 ppm and above. By 13 days, all animals in these groups had demonstrable anti-TDI IgG. In this study, 0.02 ppm represents a threshold above which both pulmonary and immune responses were observed. Although these responses were not seen when the induction dose was 0.02 ppm, once the animals had been sensitized by higher induction doses, a challenge exposure to 0.02 ppm was sufficient to elicit pulmonary responses.

In a protocol similar to Aoyama's above, Karol (1983) exposed guinea pigs to 0.02, 0.12, 0.36, 0.61, 0.93, 4.70, 7.60, or 10 ppm TDI and, beginning on day 22, examined animals for production of TDI-specific antibodies, and for dermal and pulmonary sensitivity to TDI. TDI-specific antibodies were found with exposures of 0.36 ppm and above with the antibody titers reflecting a dose-dependent increase through 0.93 ppm. Respiration rates were observed to decrease in a dose-dependent fashion during the sensitizing exposure to TDI. At exposures of 0.61 ppm and above, respiratory rates were depressed at least 50% after one hour. However, upon subsequent inhalation challenge with 1% TDI-guinea pig serum albumin conjugate, pulmonary responsiveness was measured as an increase in respiration rate. Similar to the results for antibody production, pulmonary effects were only observed in animals sensitized with 0.36 ppm TDI and above. The pulmonary effects correlated to the presence of antibodies, but not to their titer.

Pauluhn (2014) developed a respiratory sensitization/elicitation protocol in Brown Norway rats to determine a threshold dose of TDI for elicitation of asthma-like responses in sensitized, re-challenged rats. The focus of the study was to duplicate at least some phenotypes typical of diisocyanate-asthma using two cutaneous exposures to induce and boost systemic sensitization. Pauluhn and Poole (2011) found that skin-sensitization with MDI produced a more pronounced subsequent response upon inhalation challenge with MDI as compared to repeated inhalation-only sensitization, so a similar protocol was used for TDI.

Pauluhn (2014) notes that both the priming response and the elicitation response are linked to irritation/inflammation of the susceptible lung airway tissue. Thus, the dose must be high enough to overcome the scrubbing ability of peptides and proteins in lung lining fluid of the upper airways and reach susceptible lung structures in the posterior airways. As measured by changes in tidal volume that are a result of stimulation of the C-fiber-related alveolar Paintal reflex, this TDI dose was determined to be about 81 mg/m^3 (11.4 ppm) or higher. Neutrophilic granulocytes (PMNs) in BAL fluid were used as the endpoint for allergic pulmonary inflammation in the rats. This was supplemented by physiological

measurements characterizing nocturnal asthma-like responses and increased nitric oxide in exhaled breath.

A *C* x *t* regimen in which concentration (*C*) was held constant and time (*t*) was variable yielded the best dose-response relationship for the dermally-sensitized rats as long as *C* was high enough to overcome the scrubbing capacity of the upper airways (Pauluhn, 2014). In rats that were primed with three previous exposures to 85 mg/m^3 (12 ppm)TDI for 30 min each, the author identified a NOAEL of 1000 mg TDI/m^3 × min for TDI-induced asthma in rats using his *C* x *t* (in which *t* = 10, 30 or 60 min) escalation challenge protocol.

In their review of animal models for TDI airway effects, Schupp and Collins (2012) conclude that respiratory irritation and sensitization may be interdependent, and both irritation and sensitization by TDI are threshold phenomena. Animal species investigated and summarized in this review were primarily guinea pigs and mice, but it included a few studies in rats and one in rabbits. Multiple factors affect the thresholds, including diisocyanate potency, route of exposure, the extent, duration and frequency of exposure as well as other factors including genetic susceptibility and other underlying disease conditions. Nevertheless, the majority of the animal NOAELs for respiratory sensitization were in the range of 5 to 30 ppb, whereas the LOAELs were about 20 to 400 ppb. Respiratory irritation NOAELs ranged mostly from 5 to 260 ppb, whereas the LOAELs ranged from 10 to 3100 ppb. The authors concluded that the NOAELs and LOAELS for both irritation and sensitization are in the same order of magnitude across species.

6. Chronic Toxicity of Toluene Diisocyanate

Isocyanate exposure, including TDI exposure, is one of the leading causes of occupational asthma, characterized by bronchial inflammation with lymphocytic infiltration and eosinophilia, airway hyperresponsiveness, and airway remodeling (Chan-Yeung, 1990). In clinical investigations carried out by Baur et al. (1994) detailed evaluation of case histories and clinical data of 621 isocyanate workers, 247 of whom reported symptoms, showed that the predominant diagnosis was bronchial asthma followed by chronic bronchitis, rhinitis, conjunctivitis, and several other less common disorders including allergic alveolitis. Another pulmonary endpoint investigated by many researchers is an accelerated decline in pulmonary function (such as decreased FEV_1) with chronic TDI exposure in the absence of occupational asthma.

6.1. Chronic Toxicity to Adult Humans

6.1.1. Pulmonary Function as Measured by FEV_1

FEV_1 is one of the most common pulmonary function tests examined in occupational studies. It is helpful to review typical FEV_1 loss in worker and general populations first before comparing pulmonary function decrements in

diisocyanate worker populations. In healthy adults, FEV_1 has been found to decline at a rate of about 25 ml/yr (Anees et al., 2006). In asthmatic subjects and smokers with chronic obstructive pulmonary disease, declines of about 40 ml/yr and 60 ml/yr, respectively, have been found. Examined longitudinally, the Six Cities Study observed individual rates of FEV_1 loss increased more rapidly with age in never-smoking adults (Ware et al., 1990). Their longitudinal model gave rates of loss in males increasing from 16.9 ml/yr at age 25-29 to 58.0 ml/yr at age 75-78. In females, rates of loss increased from 14.6 ml/yr at age 25-29 to 41.7 ml/yr at age 75-78.

In a large steelworker population of 475 participants, smoking, being overweight, excess weight gain over time, and dust exposure at work were all related to a lower level (as measured cross-sectionally) and a steeper rate of decline of FEV_1 loss (as measured longitudinally) of pulmonary function (Wang et al., 1996). In this worker group (age at midway point 40 yrs), FEV_1 loss examined longitudinally in current-, ex- and never-smokers was 53, 44, and 37 ml/yr, respectively. In a 15-year follow-up study of a general population, the unadjusted decline in FEV_1 among subjects with asthma was 38 ml/yr, as compared with 22 ml/yr in those without asthma (Lange et al., 1998).

6.1.2. Prevalence and Characteristics of Diisocyanate-Induced Asthma

The prevalence of occupational asthma due to diisocyanates was estimated by Baur (1990) to be anywhere between 0 (seat production of a car manufacturer with no detectable TDI air concentration) and 30% (car equipment plant atmosphere with a permanent TDI concentration of 5-10 ppb). In occupational studies where TWA TDI concentrations were kept below 5 ppb, the prevalence of asthma was generally below 1% (Ott et al., 2003).

Exposure to TDI or other diisocyanates in workers with diisocyanate-induced asthma may result in an immediate or delayed asthmatic symptom onset, or have a dual or recurrent character. Diisocyanate challenges in diisocyanate-sensitized workers do not always correlate with nonspecific bronchial hyperactivity as evaluated by the methacholine challenge test. For example, of 132 workers with an asthmatic response to methacholine 71% did not respond to diisocyanates, whereas 16% of those without methacholine hyperreactivity were positive in the diisocyanate challenge test (Baur et al., 1994). Alternatively, Karol et al. (1994) found that airways hyperresponsiveness to methacholine in TDI-sensitized workers is a strong predictor of response to TDI provocation challenge, independent of atopy and serum IgE, and that serum IgE is associated with early-onset responses to TDI provocation challenge.

Most studies find no evidence that atopy or smoking influences susceptibility to diisocyanate-induced asthma (Malo et al., 1992; Baur et al., 1994). However, a case-referent study of TDI workers found smoking or history of either hay fever, eczema, or asthma increased the risk of developing TDI-related asthma 2-3-fold (Meredith et al., 2000).

It has been proposed that brief episodes of high exposure are more likely to lead to diisocyanate asthma than long-term exposure to lower concentrations (Musk et al., 1988). Thus, many researchers recorded average TDI concentrations as well as short-term peak exposures, most often defined as time spent at or above 20 ppb. However, it is still unclear what the relative importance of short-term high exposures and low, long-term exposures are in the development of diisocyanate-induced occupational asthma. Some studies found a stronger association with continuous-type exposure leading to asthma compared to short-term high exposures (Diem et al., 1982; Meredith et al., 2000).

The persistence of pulmonary symptoms for months to years following cessation of diisocyanate exposure is not uncommon (Paggiaro et al., 1990; Paggiaro et al., 1993; Piirila et al., 2008). Follow-up studies of patients with diisocyanate induced asthma resulting from TDI exposure typically find mild to moderate inflammatory responses, as indicated by elevated numbers of lymphocytes, eosinophils, and neutrophils in the bronchial submucosa and bronchial lavage fluid, along with epithelial damage and thickening of the basement membrane (Paggiaro et al., 1990). In a long-term follow-up (11 years) study, asthma-like symptoms, bronchial hyperresponsiveness and airway obstruction improved, but did not normalize with cessation or reduction in TDI exposure (Talini et al., 2013). Improvement mainly occurred in subjects with an early diagnosis of occupational asthma and in patients with a lower baseline FEV_1 no longer exposed to TDI.

Specific inhalation challenge has been referred to as the “gold standard” for diagnosis of probable diisocyanate asthma, since clinical history, questionnaires and physiological studies are frequently not definitive by themselves (Banks et al., 1996; Redlich et al., 2007). Concentrations of TDI in these challenge tests usually start at 5 ppb and increase to a maximum of 20 ppb, or until a positive challenge test is reached. In these challenge protocols, if an individual does not respond at 20 ppb exposures, it is assumed they are not isocyanate sensitized. With a few exceptions, concentrations of TDI higher than 20 ppb are not used in these challenge tests due to the potential to provoke acute allergic sensitization, sensory irritation and respiratory inflammation.

6.1.3. Latency Period for Onset of Isocyanate-Induced Symptoms

In a study of 60 workers with isocyanate-induced asthma (predominantly to TDI), the average duration of exposure to isocyanates ranged between 8 and 15 years (Mapp et al., 1988). The average duration of symptoms for these subjects before diagnosis was between two and five years showing that diagnosis was often delayed, but also that there can be a prolonged latent period between exposure and onset of respiratory symptoms. In a more detailed study of the time of exposure before onset of occupational asthma, approximately 20% of 107 subjects with isocyanate-induced asthma (principally HDI followed by MDI and TDI) had symptoms within the first year of exposure (Malo et al., 1992). Nearly 60% of subjects exposed became symptomatic after 5 years of exposure, with a

mean latency period of 7.34 years between the start of exposure and the onset of symptoms.

In a case-referent study by Meredith et al. (2000), symptoms began in 11 of 27 workers (41%) in the first year of employment at a TDI plant, with nine occurring within 3 months. The median duration employed at the time symptoms of asthma developed was 30 months. The difference between cases and referents in mean 8-hr TWA exposure was most pronounced in these 11 matched sets (1.8 ppb for the early-onset asthmatics and 1.3 ppb for asymptomatic referents performing similar jobs). No difference in peak exposures between cases and referents was found. Also, there seemed to be no association between current exposure to TDI and the development of asthma more than one year from first employment. This finding suggested to the authors that the etiology of asthma due to diisocyanates which occurs soon after exposure may differ from asthma which develops after longer periods of employment.

6.1.4. Measurement of Airborne TDI

Quantitative analysis of airborne TDI in occupational settings is challenging due to low and variable concentrations. Direct reading instruments used primarily in earlier occupational settings, such as paper tape instruments, can be used for real-time monitoring of TDI vapors but are problematic with mixed atmospheres and are not as accurate as quantitative sampling (Redlich et al., 2007). If exposures are only to the TDI monomers, then sampling will be almost exclusively for TDI vapor.

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 is influenced by factors such as the expected diisocyanate 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 adsorbant, 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 diisocyanate 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 diisocyanates, or added later to a collected sample.

After the sample has been derivatized, its components are separated for identification of individual compounds within the sample. This is most often accomplished by reversed-phase high-performance liquid chromatography (RP-HPLC). 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.

Numerous methods exist to suit a variety of researcher needs. In general, NIOSH Method 5525 may offer the most specificity, sensitivity, and applicability. 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 (NIOSH, 1998) While the filter collects particles of all sizes, it most efficiently collects and derivatizes small particulates (≤2 µm). The impinger traps diisocyanate vapors and larger 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 diisocyanates. The limit of detection by fluorescence and ultraviolet detection are 0.8 ppt and 0.08 ppb, respectively. 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).

6.1.5. Principal Occupational Studies

Longitudinal studies are the primary means for assessing asthma onset prevalence and changes in pulmonary function with time in diisocyanate workers. The following summarized longitudinal studies represent the most comprehensive reports that included both TDI exposure data and the subsequent pulmonary effects. A few selected cross-sectional studies relevant for REL determination are also summarized. The longitudinal studies are also summarized in a table format (Table 14) at the end of this Section.

Diem et al., 1982; Weill et al., 1981

The chronic REL is based on a prospective occupational study by Diem et al. (1982) of 277 male workers in a new TDI production plant. This study has several strengths over other workplace investigations of TDI exposure, including minimal co-exposure to other irritating chemicals, extensive use of personal exposure monitoring devices, accounting of TDI-sensitized workers in the cohort, and in particular, detailed longitudinal analysis of workers from the start of exposure in a new TDI production facility.

Changes in pulmonary function, measured as changes in FEV_1, were assessed with nine examinations conducted over a five year period. Baseline pulmonary function was established six months prior to the start of TDI production in 168 workers with no previously reported TDI exposure. Personal 8-hr exposures were measured with continuous tape monitors (MCM Type 4000), but not until two years into the study. A total of 2,093 personal samples from 143 workers were collected. The 8-hr TWA ranged from a minimum of 0.1 ppb to a maximum of 25 ppb, with a geometric mean of 2.00 ppb and a geometric standard deviation of 2.94 ppb.

Workers were divided into two groups. The low exposure group comprised those exposed at or below 68.2 ppb-months (which is the cumulative exposure of a worker exposed to a geometric mean TDI level of 1.1 ppb for the entire 62 months of the study), while the high exposure group comprised those above this level. In the high exposure group, the 8-hr TWA concentration of TDI was above 5 ppb for 15% of the time. A further sub-grouping was based on smoking history (never, previous, current). The arithmetic mean exposure level for the non-smokers was 1.9 ppb TDI in the high-exposure group and 0.9 ppb TDI in the low-exposure group (calculated by Hughes (1993) as cited by U.S. EPA (1995)). The higher exposure group was further limited to those individuals who showed normal FEV_1 levels (i.e. FEV_1 to height ratio: FEV_1/ht^3). This was done since it has been observed that FEV_1 level is inversely related to previous annual decline in FEV_1. Use of non-normal FEV_1 levels could result in spurious associations between annual FEV_1 decline and TDI. Data were analyzed by the maximum likelihood weighted regression approach to account for inter-individual variability in the precision of the measurements (Diem and Liukkonen, 1988).

Prevalence of bronchitis and dyspnea increased from pre-exposure baseline in the high exposure category, as measured by cumulative exposure, to a greater extent than in the low category. However, these differences in symptom increases between low and high exposure categories were not statistically significant.

Worker surveillance for the onset of TDI sensitization was presented in Weill et al. (1981). Of 277 workers in the study population, 12 men (4.3%) became clinically “sensitive” to TDI during the study. Workers were identified as clinically “sensitive” if they developed recurrent respiratory signs and symptoms upon repeated exposure to low concentrations of TDI. The definition was qualified because some workers were described as developing reversible airways obstruction in the TDI area. They obtained relief by transferring to other areas, but failed to react when challenged to TDI vapor. Nine of these 12 men became sensitized after less than 12 months of TDI exposure; eight of those nine men were sensitized after less than four months of exposure. The incidence of sensitization over the five years of the study was 0.9% per year. Six of the sensitive workers underwent bronchoprovocation challenge in a laboratory setting at Tulane University with 15 min TDI exposures of 0, 5, 10 and 20 ppb on successive days; two of these reacted with a >20% drop in FEV_1 while the other four did not.

In data provided by Weill et al. (1981), job positions held by the workers were stratified into three categories of TWA TDI exposure intensities of 6.8, 3.2 and 1.6 ppb. By this criterion 9 of 12 workers who became sensitized were in the high or moderate exposure groups. Two other workers that became sensitized worked in low TDI exposure jobs. The last sensitized worker was said to be exposed to high TDI levels in laboratory work that was not measured during the study. Six of the 12 workers had known major exposure in TDI spills, but the report did not specify which workers were exposed. Workers in high and

moderate exposure jobs were exposed to >5 ppb TDI for at least 24% of the time. Workers in the low exposure jobs were exposed to >5 ppb TDI for only 3% of the time. It was not stated specifically which exposure group the sensitized workers belonged to when stratified by two cumulative exposure groups (i.e., ≤68.2 ppb-months group or >68.2 ppb-months group). However, the authors reported that the sensitized workers likely did not affect the overall pulmonary function results.

Linear regression analysis did not find a relationship between a decline in FEV_1 and TDI exposure when TDI exposure was treated as a continuous variable. Annual change in FEV_1 was found to be significantly related to pack-yrs of smoking ($p<0.01$). Table 4 presents the annual change in FEV_1 dichotomized by three smoking categories and two cumulative exposure categories after adjusting for mean age and FEV_1 level. The mean age used for FEV_1 adjustment was 35.6 yrs and assumed a 5.8-ml increase in FEV_1 annual decline per decade of age. Among workers who never smoked, FEV_1 was significantly reduced by 38 ml/year in the high-exposure group compared to the low-exposure group ($p = 0.001$). However, among current and previous smokers, the decline in FEV_1 was not statistically significant. Comparing within the low exposure group, current smokers showed a mean FEV_1 decline of 27 ml/year greater than never smokers ($p = 0.004$).

Table 4. Annual Average FEV_1 Change by Smoking and Cumulative Exposure[a]

Cigarette Smoking	Cumulative TDI Exposure		Annual FEV_1 decline	*p* (one-tailed)
	≤68.2 ppb-months	>68.2 ppb-months		
Never	1 ml/yr (n=35)	-37 ml/yr (n=21)	-38 ml/yr	0.001
Previously	-12 ml/yr (n=31)	-15 ml/yr (n=16)	-3 ml/yr	ND[b]
Currently	-26 ml/yr (n=64)	-37 ml/yr (n=35)	-11 ml/yr	0.10

[a]Based on 202 workers with $FEV_1/height^3 \geq 550$ in Table 7 of Diem et al. (1982). FEV_1 values adjusted for mean age of 35.6 yr in order to account for differences in age distributions within cells, and a 5.8 ml increase in FEV_1 annual decline per decade of age.
[b]Statistical result was not presented but was not statistically significant ($p>0.05$)

Similarly, in the low-exposure group, the mean annual decline in forced expiratory flow (25-75%) [$FEF_{(25-75\%)}$] was 81 ml/sec-yr greater among current smokers than among never-smokers ($p = 0.003$). However, in the high exposure group, there was no effect of smoking history. For never-smokers, the mean annual decline in $FEF_{(25-75\%)}$ was 113 ml/sec-yr greater in the high exposure group than in the low-exposure group. Thus the combined effects of smoking and TDI exposure were more pronounced in the low TDI exposure group.

The association between FEV_1 annual change and time above 20 ppb dichotomized at 0.19 months was similar to that obtained using the cumulative exposure dichotomized at 68.2 ppb-months. Among never-smokers, FEV_1 was statistically significantly reduced (p=0.033) by 24 ml/year in the high-exposure group (>0.19 mo above 20 ppb) compared to the low-exposure group (≤0.19 mo above 20 ppb).

This study suggests that an annual FEV_1 and an FEF_{25-75} decline in excess of that due to aging occurred in the high exposure group. A limitation of this study is that no unexposed reference group was included, although differences in FEV_1 were observed between low and high cumulative exposure groups.

Peters and coworkers

In one of the first studies to examine the longitudinal effects of TDI exposure on pulmonary function, Peters et al. (1968) published a series of reports following a group of polyurethane workers over three years. In the first study, the pulmonary function of 38 workers was examined at the beginning and end of the work day (Monday). Significant decrements in FVC (-190 ml, p<0.001) and FEV_1 (-220 ml, p<0.001) occurred over the work day, with both nonsmokers and smokers showing similar responses. Workers with respiratory symptoms of cough and phlegm showed greater decreases in FEV_1 than workers without symptoms.

Area exposures were determined at the time of the study by a colorimetric method called the Marcali method. Individual exposure to TDI was not assessed. The limit of detection was said to be 0.1 ppb when air was sampled for 1 hour. Exposures ranged from 0.1 to 3.0 ppb, but had been 20-30 ppb in the polyurethane pouring area the previous year before ventilation was improved. Worker exposure duration ranged from 2 weeks to more than 10 years, with a mean of about 2 years.

In a six month follow-up, 28 of the workers were again examined with the same pulmonary function tests (Peters et al., 1969). The average FEV_1 change for the group compared to six months earlier was -140 ml (annual average of -280 ml/yr). The limit of detection using the Marcali method was said to be 0.5 ppb with long sampling times. At the time of the follow-up, the TDI concentration was 9-12 ppb (n=2) in the pouring area and 4-5 ppb (n=2) near the stripping molds.

The average FEV_1 change for never-smokers (-160 ml/yr, n=11) was less than ever-smokers (-360 ml/yr, n=17), but not statistically significantly different. The authors indicated larger sample sizes are needed to properly assess smoking differences. No significant correlation was found between current smoking habits and any ventilatory function. However, both lifetime packs and duration of smoking correlated significantly with one-day and six-month changes in FEV_1.

There was also a significant correlation coefficient (r=0.72) between one-day changes in FEV_1 (measured six-months earlier) and six month changes in FEV_1.

In addition, workers with respiratory symptoms (cough and/or phlegm) demonstrated greater falls in FEV_1 than did asymptomatic workers. Those workers with sputum in the morning and afternoon (n=8) had an annual average FEV_1 change of -760 ml/yr. The mean annual average FEV_1 change for the other 17 workers was -100 ml/yr.

One- and two-year follow up of the workers found a change in FEV_1 of -120 ml at one year in the remaining 25 workers, and a change of -220 ml (annual average of -110 ml/yr) at two years in 20 remaining workers (Peters, 1970). Further information on TDI exposure and worker condition was not provided with the exception that the correlation for one-day changes in FEV_1 (before and after Monday work) with one year changes was still strong (r=0.71). The only data presented at the end of year three of the study showed that the decrement in FEV_1 was still persistent in the workers (number unknown) at 120 ml/yr (Peters, 1974).

A more detailed report of the 18-month follow-up was conducted by Peters et al. (1970). FEV_1 was measured in the 19 remaining workers common to the first survey. The cumulative change in FEV_1 was -220 ml, or -147 ml/yr. This change was statistically significant ($p<0.02$) and exceeded the expected change associated with aging and smoking. It was unclear from the study how many of the remaining workers were asymptomatic for respiratory effects of TDI. Sampling using the Marcali method found TDI concentrations of 3.0 to 14.5 ppb in the pouring area (n=9 samples), and concentrations at or below the limit of detection (0.5 ppb) to 2.0 ppb in other areas of the facility (n=14 samples).

Table 5 presents the overall summary of changes in FEV_1 found in the workers over the three years of the study.

Table 5. Mean change in FEV_1 in polyurethane workers over three years of study by Peters and coworkers

Follow-up period	Annual average change in FEV_1 (ml/yr)	Number of workers	*p*-value
6 mo	-280	28	<0.02
1 yr	-120	25	<0.02
1.5 yr	-147	19	<0.02
2 yr	-110	20	<0.01
3 yr	-120	*	*

* Data not presented

This study is limited by use of area exposure sampling with a method (i.e., Marcali) that may seriously underestimate the true TDI exposure at polyurethane plants (Rando et al., 1984; Omae et al., 1992b). This colorimetric technique measures only total TDI concentration, but the signal strength is different for the two TDI isomers. The 2,6-TDI isomer gives about a 55% lower response than 2,4-TDI isomer. In occupational settings, use of high performance liquid

chromatography determined that 2,4-TDI is more efficiently reacted in the polymerization process. Thus, a starting solution of 80% 2,4-TDI and 20% 2,6-TDI has been shown to produce a contaminated air concentration dominated by the 2,6-TDI isomer during end stages of polyurethane production. If the Marcali method was based on the signal given by the starting solution dominated by 2,4-TDI, as was probably the case, the air concentration of TDI will likely be underestimated.

Other limitations include lack of a control worker cohort, although the authors stated no FEV_1 changes were found when the authors tested themselves (Peters et al., 1969). The statistical analysis by the authors included workers experiencing respiratory symptoms. However, Peters (1974) noted that although many of the workers experienced cough and phlegm, none experienced symptoms of chest tightness or shortness of breath from the TDI exposures. This finding would suggest that many of the workers had chronic bronchitis. Finally, no follow-up of the workers that left the study early was conducted.

Wegman and coworkers

In 1972 Wegman et al. (1974) examined 112 polyurethane workers exposed to TDI for acute pulmonary function changes during the work shift on the first day of the work week. A follow-up of 57 remaining original workers was conducted two years later to investigate longitudinal pulmonary function changes (Wegman et al., 1977). Comparison of the retested and lost workers did not find any selection bias. Sampling included both area and personal sampling using the Marcali method, the sensitivity of which allowed the detection of TDI at 0.5 ppb. A total of 118 personal and 14 area samples were taken to characterize 20 work stations over the course of the two-year study. Ventilatory capacity was assessed on Monday morning following a three-day weekend.

The average exposure values by job ranged from 0.5 to 9 ppb TDI. To examine the results for a dose-effect relationship, three exposure categories were created, ≤1.5 ppb, 2-3 ppb, and ≥3.5 ppb TDI. Reports of symptoms collected on a standard questionnaire found the prevalence of cough and phlegm increased proportionally with increase in exposure. About 15% had responses consistent with chronic bronchitis (cough or phlegm for most days for three months of the year) but not associated with exposure. Thirty percent reported wheezing occasionally or most of the time, and 10% reported dyspnea, but neither was associated with exposure. OEHHA notes that these symptom findings suggest sensitization to TDI in some workers, but it was not investigated by the authors.

Table 6 presents the annual average change in FEV_1 over the two-year period of the study. Analysis of Variance (ANOVA) showed a significant difference in loss of FEV_1 according to exposure groupings ($F = 5.2539$, $p<0.01$). The study authors report that possible confounding variables including age, months employed, smoking habits and lung size did not explain the differences.

Table 6. Two-year change in FEV_1 1972-1974 and annual average change in FEV_1

Variable	Exposure Concentration (ppb)		
	≤ 1.5 (n=20)	2-3 (n=17)	≥ 3.5 (n=20)
Months in plant	51.5	55.8	55.1
# Nonsmokers	4	2	4
2-yr Δ FEV_1 in ml, mean (±SD)[a]	-12 (204)	-85 (177)	-205 (185)
FEV_1 in ml/yr	-6	-43	-103

[a] FEV_1 not corrected for normal decline in FEV_1 with age; SD = standard deviation

A follow-up examination was carried out on a Monday before work of the remaining workers (n=48) two years later in 1976 (Wegman et al., 1982). Sixty-nine personal and area samples were collected on the day of pulmonary function testing and employed the Marcali method as before to estimate TDI concentration. Personnel records were used to estimate exposure to each worker during both the 1972-1974 and 1974-1976 time intervals. Accurate information on exposure history and acceptable spirograms were obtained for 37 of the 48 workers.

Overall median TDI concentration for 1974-1976 was 3 ppb; the job identified as mixing and pouring has the highest median exposure of 7 ppb (range: 5 to 40 ppb). The population was divided into exposure categories of low (<2.0 ppb), medium (2.0-3.4 ppb), and high (>3.5 ppb) exposure.

Upper and lower respiratory symptoms were found to be unrelated to exposure category. The four-year change in FEV_1 shown in Table 7 was statistically significantly related to exposure category by the ANOVA (p=0.007, F statistic not given), with the t-test showing a difference between the low and high exposure groups (p=0.002).

Table 7. Four-year change in FEV_1 1972-1976 and annual average change in FEV_1.

Variable	Exposure Concentration (ppb)		
	≤ 2.0 (n=13)	2.0-3.4 (n=13)	≥ 3.5 (n=11)
Months in plant	82.5	81.1	67.0
# Nonsmokers	4	2	3
4-yr Δ FEV_1 in ml, mean (±SD) [a]	+2 (168)	-133 (184)	-242 (174)[b]
FEV_1 (ml/yr)	~0	-33	-60

[a] FEV_1 not corrected for normal decline in FEV_1 with age; SD = standard deviation
[b] ANOVA f, p=0.007; t-test low vs. high, p=0.002

Analysis of possible confounding variables including age, months employed, smoking habits and lung size did not explain the differences. Comparison of the

survivor group with the initial cohort indicated that the restudied group was representative of the original group from 1972. Thus no selection bias was introduced due to loss of subjects from the study.

These studies are limited by use of the Marcali method that may have considerably underestimated the true TDI exposure. OEHHA notes that the authors did not discuss the potential for TDI-sensitive workers being included in the analysis of longitudinal change in FEV_1. Also, sample size of the groups is small largely due to high attrition rates. Diem et al. (1982) also suggests it is difficult to estimate true annual FEV_1 change from only three data points. The authors state that smoking had no effect on FEV_1, although this may have been a result of the small sample sizes.

Musk and coworkers

Analysis of FEV_1 and respiratory symptoms was conducted in 259 workers at two polyurethane plants in 1971, with 107 available for follow-up examination five years later (Musk et al., 1982; Musk et al., 1985). Over the five years of the study, 2,573 samples were collected by hand-held devices in breathing zone areas of subjects employed in the polyurethane pouring area. Samples were also collected at other sites during the survey where highest exposures were encountered. The sampling time was 20 to 60 min and analysis was done by the Marcali method. Mean geometric TDI exposures over the last 4 years of the study were 1.5 ppb at Plant 1 and 1.0 ppb at Plant 2. The 90^{th} percentile concentration was 5.0 ppb at Plant 1 and 3.6 ppb at Plant 2. Very low geometric mean concentrations of MDI were also present (0.3 and 0.6 ppb).

No workers described acute exposure-related symptoms with exposure to TDI. Bronchitis was found to be more prevalent in smokers. The mean annual decline in FEV_1 for all subjects was 20 ml/yr, which was concluded by the authors to approximate normal aging declines. For workers with no exposure to isocyanates (n=42), the annual average decline in FEV_1 was 22 ml/yr. The average annual decline in FEV_1 for those exposed only to TDI (n=17) was 26 ml/yr and not statistically significantly different from non-exposed workers. In addition, no excess decline in FEV_1 was reported for those workers exposed to MDI (n=25) or to both TDI and MDI (n=6). Stepwise regression analysis showed the 5 yr decrement in FEV_1 was significantly related only to current smoking. Workers that had been lost to follow-up had similar lung function to those who remained in the study.

A ten-year follow-up was carried out in 42 remaining workers by Gee and Morgan (1985) in 1981. A group of 12 additional workers at the plants with no isocyanate exposure was used as a control group. The authors indicated the rate of decline of ventilatory capacity could not be accurately calculated since many of the 1971 measurements were not valid. This assertion had been refuted by Musk et al. (1985). Nonetheless, Gee and Morgan (1985) did not detect an excess annual FEV_1 decline in the exposed workers. Follow-up of exposed workers that had left

employment was not conducted, although the authors noted no worker compensation claims had been filed.

Omae, 1984

Pulmonary effects were investigated in 106 workers at TDI production plants and 39 referents in 1980, and in 64 workers and 21 referents on the 2-year follow-up in 1982 (Omae, 1984). The workers wore personal MCM 4000 paper-tape monitors for TDI during working hours. The numbers of samples collected from the followed workers were 161 in 1980 and 106 in 1982. The mean duration of TDI exposure was 9.0 years in 1980 and 11.2 years in 1982.

Arithmetic means of 8-hour TWA exposure concentrations were 0.7 ppb in 1980 and 1.0 ppb in 1982. Because the TDI process was automated, the workers spent much of their working time in clean areas. The author indicated exposures occurred only during excursion into TDI areas. As a result, only two of the TWA exposure concentrations exceeded 20 ppb. Short-term concentrations (presumably 15 minutes) exceeding 20 ppb occurred in 15 of the 161 samples in 1980 and 2 of 106 samples in 1982.

No statistically significant differences were observed in the pulmonary function between the TDI workers and the referents, or in two-year reductions in FEV_1 or FVC between TDI workers and the referents. The change in FEV_1 was -50 ml/yr among exposed and -65 ml/yr among the referents. The change in FVC was -25 ml/yr among exposed and -40 ml/yr among the referents. Eight workers had exhibited episodes of acute asthmatic reactions soon after having begun their TDI jobs (mean exposure duration 11.7 yrs) but had continued working at the plants. The authors imply these workers developed asthma prior to the start of the study in 1980 when TDI exposure was higher. Specific TDI sensitivity was not assessed in these workers. Although some of these individuals did show decreased pulmonary function (in particular, peak expiratory flow), the two-year decrement in pulmonary function in the asthmatic workers was not different from that in the other TDI workers.

The author observed that the relatively low follow-up rate could introduce biases. Reasons for losses of about 80% of TDI workers were not related to TDI exposure. However, follow-up of the remaining 20% of workers lost was not performed and appeared to fall into one of three categories: left due to plant closing, plant transfer, and absent from work on day of test. Comparison of the data in 1980 between workers successfully followed and the 42 workers lost indicated negligible differences in pulmonary parameters and that selection biases were not considered a concern.

Higher prevalence of sensory irritation was observed in the follow-up survey, but the symptoms were more often attributed to other chemical exposures. Co-exposure to other irritants included phosgene, chlorine, nitric acid and sulfuric acid, all of which are raw materials for TDI production.

Omae et al., 1992

Fifty-seven polyurethane foam manufacturing workers and 24 reference workers were followed for four years to examine the long-term effect of TDI exposure on pulmonary function (Omae et al., 1992a; Omae et al., 1992b). The workers wore personal paper-tape monitors for TDI (MCM 4000, MDA Sci.) on their chest during working hours. The numbers of samples collected from the followed workers were 59 in 1981, 48 in 1983, and 52 in 1985. The forced maximal expiratory flow-volume was examined in the same manner as in the first cross-sectional study in 1981 and the follow-up surveys. Adjustment for age and height of the forced expiratory flow-volume parameters was conducted using the prediction equations originally calculated by the authors with a group of non-exposed males.

None of these workers were considered hypersensitive to TDI. The authors noted that the polyurethane workers had been engaged in their jobs for a mean of 13.3 years and sensitized workers may have left prior to the survey. Thus, the workers examined may represent a survivor population. Greater prevalence of eye irritation during or after work, nasal stuffiness or discharge in winter, and phlegm in the morning in winter were statistically significantly greater ($p<0.05$) in non- and ex-smoking polyurethane workers than in the non- and ex-smoking reference workers.

Exposed workers were divided into two groups, workers in mold-type manufacturing processes (n=28) with low-level TDI exposure (TWA 0.1 ppb), and workers in mostly slab-type factories (n=29) where short-term exposure to TDI often exceeded 20 ppb. The slab-type polyurethane foam workers were further divided into 2 subgroups, one subgroup consisted of workers (n=15) exposed to mean and maximal TWA concentrations of 8.2 and 30 ppb, respectively, and the other subgroup (n=14) was exposed to lower mean and maximal TWA concentrations of 1.7 and 4 ppb, respectively. The ranges of peak exposure levels for the high and low slab-type polyurethane workers were 30-80 ppb and 3-14 ppb, respectively.

No differences were observed between the four exposure groups for average annual loss of FVC, MEF_{50} (forced expiratory flow at 50% FVC), and FEV_1. However, greater than expected average annual loss in other flow-volume indices was observed (Table 8). For the high exposed slab-type polyurethane workers, greater than expected average annual losses ($p<0.05$) occurred for MMF (maximal mid-expiratory flow), FEV_1% (FEV_1/FVCx100), and MEF_{25} (forced expiratory flow at 25% FVC). The average annual loss in these three indices was also greater in the high exposed slab-type workers compared to workers exposed in mold-type factories ($p<0.05$). Finally, the high exposure slab-type workers also showed a greater average annual loss in PEF (peak expiratory flow) compared to the reference workers. For the lower exposed slab-type polyurethane workers, greater than expected average annual losses ($p<0.05$) occurred for MMF and MEF_{25}.

Table 8. Statistically significant average annual loss indices among high-exposed and low-exposed slab-type workers, mold-type workers and reference workers.

Variable	Worker Exposure Group (mean ± SD)			
	Slab workers High exposure (n=15)	Slab workers Low exposure (n=14)	Mold workers (n=28)	Reference workers (n=24)
TWA Exposure	8.2 ppb	1.7 ppb	0.1 ppb	0 ppb
MMF	-2.39±4.13[a]	1.91±3.02[b]	0.11±2.96	-0.30±2.39
FEV_1 %	-0.89±0.96[a]	0.19±0.63	-0.10±0.94	-0.29±0.88
MEF_{25}	-3.28±5.64[a]	3.38±4.39[b]	1.01±4.37	-0.50±5.49
PEF	-0.38±1.46[c]	0.66±1.79	0.61±1.61	0.72±1.66

[a] $p<0.05$ against expected value of average annual loss and mold workers
[b] $p<0.05$ against expected value of average annual loss
[c] $p<0.05$ against reference workers

The average annual loss of pulmonary function over 4 years was larger in smokers than in non-smokers, although the differences were not significant. The lack of an effect due to smoking, the authors noted, was probably because the number of subjects was too small to detect significant differences. The lack of differences in annual average loss between the low exposed slab-type workers compared to reference workers suggested to the authors that TWA concentrations of 1.7 ppb and a maximal TWA level of 4 ppb with short-term peaks up to 14 ppb may not cause long-term pulmonary function loss in those who are not hypersensitive to TDI. However, the authors concluded that pulmonary function loss occurred in workers with short-term TDI exposures above 20 ppb (i.e., 30-80 ppb) and TWA exposure to 8.2 ppb.

Co-exposure to other chemical irritants occurred in the workers during polyurethane foam manufacturing processes. Some of these chemicals include tertiary amines, organic tin compounds, polyols, silicon oil, dichloromethane, freons and flame-resistant agents.

Jones et al., 1992

Exposure to TDI was studied for effects on respiratory health of workers in two plants manufacturing polyurethane foams (Jones et al., 1992). TDI levels were measured at yearly intervals for 5 yr (1982 to 1986) at Plant 1 and for 4 yr (1982 to 1985) at Plant 2. Workplace concentrations were determined using the MCM TDI-sensitive paper-tape continuous monitoring system. A total of 258 workers wore monitors on 507 shifts producing 4,845 measurements. To improve accuracy in recording peak exposures and time-weighted averages under conditions of fluctuating TDI concentrations, a sample collection pattern of 12 min on, followed by 24 min of no sampling, was repeated during the 8-hr work shifts to produce up to 14 evenly spaced 12 min samples. The lower detection limit was 1 ppb before 1983, and 0.5 ppb from 1983 on. Samples showing no detectable TDI were assigned a value of one-half the lower detection limit (LDL).

With additional limited sampling done in the 1970s, personnel records were used to track each employee's career, assigning exposures as the TDI level in ppb times the number of months worked in specific job groupings in the plants. Cumulative exposure, in units of ppb x month, is the sum of all such doses; average exposure is cumulative exposure divided by length of employment. There were 394 workers present at the start of the study, and, through the fourth examination, a total of 435 had worked in one or the other plant. Six yearly spirometry examinations were performed on the workers to assess respiratory health and estimate rates of annual change in lung function.

Of 4,845 personal TDI monitoring measurements, 50% reached or exceeded the LDL, 9% reached or exceeded 5 ppb, and 1% exceeded 20 ppb. In a group of jobs involving exposure to foam ingredients or freshly produced foam, the respective figures were 68%≥LDL, 20%≥5 ppb, and 4%≥20 ppb. Mean TDI concentrations by specific job category ranged from 4.47 ppb for foam production (plant 2) to 1.17 ppb for "other".

At initial interview of 380 workers, a trend for increased prevalence of "chronic bronchitis" (cough or phlegm for more than 3 months yearly in the previous 2 years) with increasing cumulative exposure (hire to start of study) was observed, with prevalences in low, middle and high terciles of 2.6, 6.5, and 14.3%, respectively. Terciles refer to any of the two points that divide an ordered distribution into three parts, each containing a third of the population. The exposure terciles presumably refer to exposures of ≤32, >32 and ≤86, and >86 ppb x month presented later in the paper. Logistic regression analysis showed a significant association of prevalence with exposure ($p<0.02$) after controlling for smoking, age and sex. Mean initial lung function (percent predicted) and mean decline in FEV_1 were indicated as marginally worse in those with chronic bronchitis (data not shown). At the time of follow-up examinations of the remaining workers (n=262), 12 workers had been identified as developing TDI-sensitization, 6 of whom were tested and confirmed by TDI challenge.

When initial pulmonary function values (hire to start) were presented by exposure tercile, the percent of predicted FEF_{25-75} in the highest tercile (>86 ppb x month) showed a statistically significant reduction (Table 9). No differences were observed for FEV_1 and FVC as a result of cumulative exposure to TDI.

Table 9. Initial pulmonary values by exposure tercile

Variable	Tercile Exposure, Hire to Start (ppb x month)		
	≤ 32 (n=108-120)	> 32, ≤ 86 (n=109-121)	> 86 (n=112-121)
Years in plant	4.45±0.50[a]	6.34±0.28	17.56±0.55
FEV_1, % pred	107.4±1.1	106.3±1.2	104.2±1.4
FVC, % pred	107.7±1.1	106.1±1.0	105.1±1.2
FEF_{25-75}, % pred	88.0±2.0	91.0±2.4	82.3±2.3[b]
FEV_1/FVC, % pred	97.0±0.6	97.0±0.7	96.3±0.7

[a] All values presented as mean±standard error

[b] Difference among means: p<0.025

Mean initial FEV_1 and FVC in Table 9 were above 100% of predicted values. The reason cited by Jones et al. was the "healthy worker effect", which often results in working populations derived from the general population that exceed the health of the general population as a whole.

When cumulative TDI exposure was treated as a continuous variable (in increments of 100 ppb x month) there was a marginally significant effect (p<0.063) on FEF_{25-75} over all smoking categories. Each increment of 100 ppb x month was associated with a reduction in FEF_{25-75} of 2.3% predicted. When the effect of smoking on initial pulmonary function was examined, a significant reduction was found only in current smokers; for each 100 ppb x month increment, associated reductions of 4.3% of predicted FEV_1 value (p<0.0003) and 4.4% of predicted FVC value (p<0.0001) were observed.

Jones et al. also presented a longitudinal analysis of pulmonary function change for the workers over the five years of the study (Table 10). The observed mean annual declines were steep in relation to expected values, but no statistically significant difference was observed.

Table 10. Observed mean annual change in pulmonary function values in relation to expected values

Cigarette Smoking	Lung Function (in relation to expected values)		
	FEV_1	FVC	FEF_{25-75}
Never	-53 ml/yr	-51 ml/yr	-76 L/sec-yr
Previously	-59 ml/yr	-59 ml/yr	-71 L/sec-yr
Currently	-67 ml/yr	-66 ml/yr	-99 L/sec-yr

When annual change was studied using weighted multiple regression followed by weighted stepwise multiple regression, the authors found that neither smoking nor measures of cumulative or average exposure were significantly related to annual change. The FEV_1 change was -6 ml/yr for current vs. never smoker (p<0.3). Examining only the highest tercile (>86 ppb x month) of exposure, mean FEV_1 annual changes were virtually identical across the smoking categories: -65, -61, and -61 ml/yr in current, ex-, and never-smokers, respectively.

The authors concluded that their study showed evidence of past and ongoing adverse effects on respiratory health, but it was only attributable in part to measured and estimated TDI exposures. Although the authors discussed their confidence in the reliability of their data, they could not fully rule out misclassification of exposure or co-exposure to other workplace chemicals as contributors to effects not fully explained by TDI exposures.

Clark and coworkers

This longitudinal study represents the largest investigation of the pulmonary effects of TDI-exposed workers at low concentrations (Clark et al., 1998). A population of 780 polyurethane workers in 12 United Kingdom factories was followed for five years to determine whether longitudinal declines in ventilatory capacity and the occurrence of respiratory symptoms were related to TDI exposure. The workers were divided into three subgroups: a "high" exposed worker group that manufactured the polyurethane products, a low-exposure handling group that handled cold polyurethane after manufacture, and a control group of factory office workers with minimal background exposure. During the study, 88 workers left. The average time in the study for the remaining 692 workers was 4.3 yrs.

Continuous tape monitors (MCM type 4000) were used throughout the study and were capable of measuring TDI concentrations between 1 and 40 ppb. A total of 2,294 measurements were collected. The UK 8-hr (5.8 ppb) and 15-min (20 ppb) maximum exposure limits were exceeded in 4.7% and 19% of the samples taken, respectively. The short-term exceedances were all from the high exposed group. In all, 8.8% of the observed peak measurements were at or above the upper level of detection in excess of 40 ppb. Although MCM monitors are used for short-term exposure estimates, these monitors have a minimum resolution time of 9-10 min for TDI. This duration may underestimate very short duration peaks. A portable ion-mobility spectrometer with a rapid response time (on the order of a few seconds) was used post-study to monitor the peak concentrations from some of the manufacturing processes. Transient TDI peaks of up to 200 ppb were recorded, which equated to MCM-measured peaks of up to 30 ppb.

The high-exposed group of 521 workers had an in-study mean daily cumulative exposure of 9.6 ppb-hr, and an 8-hr TWA exposure of 1.2 ppb. In a summary of the industrial report by Garabrant and Levine (2000), the in-study mean daily cumulative exposure for the handler group and the office worker group was 5.0 and 2.5 ppb-hr, respectively. Based on an 8-hr workday these were said to be equivalent to average TWA daily exposures of 0.6 ppb for the handlers and 0.3 ppb for the office workers. Clark et al. noted that some HPLC samples taken in the control and handling areas showed TDI levels were below the level of detection (0.1 ppb) for this method. Given some baseline "noise" on the MCMs, which has a higher detection limit (1 ppb), the assigned exposures for such subjects may have been overestimated.

Health questionnaire responses found a statistically significant increase in wheezing in the high-exposure group ($p<0.01$) and the low-exposure handling group ($0.01<p<0.05$). Those workers who left the study from the high-exposed and low-exposed groups reported an increased incidence of breathlessness, wheeze and chest illness as compared with those not leaving. This includes 7 of 24 cases of respiratory sensitization identified during the study.

The 24 cases of TDI-sensitization identified during the course of the study were presumably from the total of 780 workers examined. Thus, 3.1% of workers became sensitized resulting in an annual incidence of 0.6% over 5 years. The published study did not indicate which exposure group these workers were in, but the industrial report by Bugler et al. (1991) and summarized by Ott (2002) indicated that 14 of 18 new hires that became sensitized after the start of surveillance had jobs with routine short-term TDI exposures above 20 ppb. Three others that became sensitized had jobs with routine short-term exposures of 10-19 ppb. The last of these 18 workers had a low exposure job, but had previous work in exposed areas with routine exposure >20 ppb. The FEV_1 decline in sensitized workers shown in Table 11 was greater than those not sensitized, but was not found to be statistically significant (p=0.29).

For the lung function measurements, linear regression analysis found no TDI-exposure related effect on FEV_1, FVC, peak flow and FEV_1/FVC% for any of the exposure groups. The mean annual decline in FEV_1 was 38 ml/yr for the exposed group of 521 workers. Mean annual declines in FEV_1 for the handler and control groups were not presented by the authors. A subgroup of male fitters and electricians in the exposed group with irregular TDI exposure, shown as maintenance workers in Table 11, did not have a different annual change in FEV_1 compared to the remaining male exposed group (p=0.25). Female workers in the exposed group (n=49) were not included in this analysis.

Linear regression analysis found a smoking-related effect on FEV_1, which was not considered statistically significant by the researchers ($0.05<p<0.1$). The smoking group represented a subset that smoked >15 cigarettes/day; their FEV_1 declined at a rate of 7 ml/yr more than that of nonsmokers (Table 11). The authors noted there was a suggestion of an increased decline in FEV_1 with increasing TDI exposure in non- and ex-smokers as compared with smokers, but was not found to be statistically significant.

For the study population the average decline in FEV_1 determined for the 88 workers who left during the study (leavers) was not significantly different from that recorded for the other workers (non-leavers) (Table 11).

Table 11. Summary of FEV_1 Annual Change Comparisons from Clark et al. (1998)

Variable	FEV_1 Annual Change
High- exposure group (n=521)	-38 ml/yr
Low-exposure (Handler) group	NS[a]
"Control" group	NS
Nonsmoker[b] (n=157)	-29 ml/yr
Smoker (n=253)	-36 ml/yr
TDI-sensitized (n=24)	-49 ml/yr
Not TDI-sensitized (n=756)	-38 ml/yr
Non-leavers (n=692)	-38 ml/yr
Leavers (n=88)	-40 ml/yr
Exposed group naïve workers (n=119)	-34.5 ml/yr
Exposed group non-naïve workers (n=402)	-39.5 ml/yr
Exposed maintenance male worker subgroup (n=95)	-34 ml/yr
Remaining exposed male workers (n=377)	-41 ml/yr

[a] NS: Not stated in the report

[b] The n represents the population of nonsmokers. It was unclear from the study if ex-smokers were included in this group or analyzed separately.

Clark et al. also examined a subgroup of workers who entered the study after the first longitudinal measurements were made and had no known prior TDI exposure (naïve workers). The naïve worker population (n=157) showed a decline in FEV_1 of 36 ml/yr and non-naïves (n=623) showed a decline in FEV_1 of 39 ml/yr. The difference was not statistically significant. Additionally, exposed naïves in Table 11 did not show a significant excess decline in FEV_1 as compared to exposed non-naïves (p=0.56).

Longitudinal regressions on the data from naïve workers show an in-study mean daily excess decline in FEV_1 (p=0.016) and FVC (p=0.026) in the early years of employment. The effect sometimes occurred immediately following the first exposure and is suggestive of an irritant response affecting those already suffering from non-specific bronchial hyperreactivity, rather than being suggestive of an allergic mechanism. It was not clear in the study if all naïves were included in the analysis, or if only those exposed to TDI during manufacturing processes were included. Further analysis of naïve subjects for more than 4 years did not demonstrate an excess decline in pulmonary function as compared to non-naïves. Thus, further deterioration did not occur in naïve workers, but neither was there recovery to pre-employment values that would indicate a "spirometry-learning effect".

A follow-up was conducted over the period of 1997-1998 on 251 workers – the majority from the original population of 780 workers (Clark et al., 2003). The authors called this a survivor population due to large attrition rates from factory closures and industry restructuring that occurred between studies. The available survey records from about 60% of all leavers indicated that respiratory illness

was the reason for leaving in 2.3% of cases. Any workers diagnosed with TDI sensitization had been removed from exposure and were no longer available in the current study. As in the previous study, workers were divided into a high-exposed (n=175), low-exposed (handling) (n=26) and low-exposure "control" (n=50) groups. Personal measurements were recorded only for the exposed group. Of the 1,004 measurements recorded as a mean cumulative exposure in ppb-hr, only 1.3% were in excess of 46.4 ppb-hr (equivalent to 5.8 ppb TDI over an 8 hr workshift), This is a reduction from the 6.9% over the period 1981-1986 observed in the previous study. Mean exposure was 8.4 ppb-hr (equivalent to 1.05 ppb TDI).

Allowing for the effects of age, gender and smoking habits, regression analysis for the high-exposed group showed no relationship between the annual losses of FEV_1 and FVC, and the mean daily exposure to TDI (Clark et al., 2003). Annual declines for the high-exposed group with a mean age of 48 yrs at the end of the study were 35 ml per year for FEV_1 and 30 ml per year for FVC. These results are similar to what was reported previously in Clark et al. (1998). The small handling group showed excess declines in both FEV_1 and FVC (data not shown in study) compared to the control subjects. The low n and the higher proportion of smokers in this group were attributed by the authors as causes for this effect. Their conclusion was that the study did not provide evidence of a TDI-related decline in FEV_1 and FVC.

Ott et al., 2000

Ott et al., (2000) conducted an analysis of employees ever assigned (n=313) to a TDI production unit at a manufacturing complex for $\geq$3 months during the period 1967 to 1992. The workers were compared to a group of frequency matched referents (n=158) without known exposure to TDI. The duration of TDI unit assignments averaged 5.7 and 4.7 years among men and women employees, respectively, with a range of 3 months to 30 years. Reports during visits to the occupational clinic of incidents related to TDI and annual periodic examination results (questionnaire, physical findings, and spirometry) were abstracted and assessed relative to industrial hygiene estimates of exposure to TDI.

Exposure estimates were initially conducted by area sampling in 1967 with a Uni-jet TDI-in-air sampler. Personal monitoring by the paper tape method (MCM4000 personal monitor) began in 1976. Starting in 1989, personal sampling for TDI was performed by OSHA method 42, which uses glass fiber filters coated with 1-(2-pyridyl)piperazine followed by solvent desorption. Analysis was by HPLC. A job-exposure matrix approach was used to estimate individual exposure to TDI. Job specific work histories were coded for each person and linked to industrial hygiene measurements. Peak exposure and TWA concentrations were aggregated on a job and time specific basis for three job groups with potentially low, medium, and high exposure to TDI. Cumulative dose estimates (ppb-months) were computed by multiplying the mean TWA concentration during

a particular job assignment by the time spent on that job (expressed in months) and summing across all job assignments.

Regression analysis showed that TWA estimates declined significantly over time for all job groups. Area sampling between 1967 and 1973 were mostly <10 ppb, with concentrations of 60 to 80 ppb in high exposure areas. Personal 8-hour samples collected by the paper tape method from 1976 to 1988 averaged 5.9 ppb TDI (n=156). Eight hour samples collected by the filter method between 1989 and 1997 averaged 2.8 ppb TDI (n=84). The TWA estimates for high exposure jobs were 9.9 ppb TDI before 1985, and <5 ppb after 1985. The average TWA concentration after 1985 across all jobs and times was 4.2 ppb TDI. Table 12 presents the distribution of the average concentration of TDI for the 313 workers over their entire work career.

Table 12. Average TDI concentration across all jobs in the TDI unit, and the number (n) and percentage of workers (%) in each exposure group.

Average TDI concentration (ppb)	n	%
<1.0	13	4.2
1.0-2.9	113	36.1
3.0-4.9	59	18.8
5.0-6.9	94	30.0
≥7.0	34	10.9

Incidents of exposure to TDI were reported by 77 different employees and included 58 incidents related to asthmatic or allergic skin reactions in 29 different workers (19 with asthmatic reactions only, nine with skin allergies only, and one person with both asthma and skin allergies). The yearly incidence of asthma induced by TDI was 1.1%, but was higher before 1980 (1.8%) than after 1979 (0.7%). The incidence for workers assigned to the TDI unit for at least 20 years was estimated to be 11.5%.

Spirometric data obtained before 1980 were judged to be unacceptable due to data quality. The average number of lung function tests per employee was 7.5 and the average interval between the earliest and most recent test was 9.3 years. Cross sectional analysis of FVC, FEV_1 and FEV_1/FVC% based on the most recent spirometry test found age, height, race, and sex were significant predictors of FVC and FEV_1. Pack years smoked was a significant risk factor for FVC, FEV_1 and FEV_1/FVC%.

The TDI concentration and cumulative dose were not significant predictive factors in the full model or in models without occupational asthma as a covariate. An additional set of analyses carried out for 119 never-smokers also found no significant relation between outcome of spirometry and either TDI concentration or dose. In addition, cross-sectional analysis was carried out with the earliest available lung function test to determine if previous exposure to TDI was

associated with lung function tests. Neither TDI concentration nor cumulative dose was significantly related to these tests. However, occurrence of previous incidents of exposure to phosgene, a chemical used in production of TDI, was associated with declines in both FEV_1 and FVC.

Longitudinal analyses were performed to estimate the annual change in FVC and FEV_1 for various subgroups of the population with ≥3 lung function tests covering an interval of ≥2 years. Declines in FVC and FEV_1 were greater among cigarette smokers than never-smokers, but cumulative dose of TDI was not significantly related to the annual decline in either FVC or FEV_1 (Table 13). The authors concluded that in agreement with other studies conducted in workplaces with exposures ranging up to 5 ppb TWA and where active medical surveillance and exposure monitoring programs were in place, there was little evidence of a relation between exposure to TDI and either FVC or FEV_1 lung function decrement. This study, however, relied on some retrospective construction of exposures using different measurement techniques; thus, exposure misclassification could be a problem.

Table 13. Estimated annual change in FEV_1 and FVC in selected subgroups

Population Subgroup	Lung Function Value	
	FEV_1	**FVC**
Men		
Exposed (n=209)	-37 ml/yr	-37 ml/yr
Referents (n=65)	-35 ml/yr	-34 ml/yr
Women		
Exposed (n=10)	-20 ml/yr	-27 ml/yr[a]
Referents (n=12)	-14 ml/yr	-14 ml/yr
Men, never smokers		
Exposed (n=67)	-31 ml/yr	-33 ml/yr
Referents (n=25)	-36 ml/yr	-34 ml/yr
Men, ≥20 cigarette pack-yrs		
Exposed (n=72)	-43 ml/yr	-44 ml/yr
Referents (n=24)	-44 ml/yr	-41 ml/yr

[a] FVC statistically different (p=0.03) from controls but referent women had more pack-yrs smoking (14.8 vs. 4.6) and follow up interval nearly 5 yrs longer for referent women.

Bodner et al., 2001

In this longitudinal study at a Texas chemical manufacturing facility from 1971 through 1997, 305 TDI manufacturing workers and 581 hydrocarbons workers never employed in isocyanate processes were examined to determine if TDI was associated with changes in respiratory measures (Bodner et al., 2001). Accurate work history records and data from routine medical surveillance examinations were used to trace changes in lung function of the workers. Workers who spent at least 3 consecutive months in TDI-related departments were selected. Personal sampling over the duration of the study included the MCM Model 4000 paper-tape method, followed by use of the colorimetric Marcali method, and

beginning in 1989, use of continuous monitors employing the colorimetric paper-tape based method. From 448 8-hour TWA TDI samples and known work histories, average TDI exposures and cumulative exposure estimates in ppb-months were determined for each work segment for each worker. Mean employment in TDI departments was 3.8 ± 4.1 yrs (SD).

Mean TDI exposures were 2.3 ppb (SD±1.0 ppb) for 8-hour TWA, and 96.9 ppb-months (SD±110.6 ppb-months) when expressed as cumulative TDI exposure per month. Maximum individual worker TDI exposure was 5.2 ppb for 8-hour TWA and 639 ppb-months. Since 1980, mean 8-hour TWA TDI exposures were at or below 3 ppb for all job categories. When exposure was expressed as cumulative TDI exposures of 1-12, 13-60, 61-120, and >120 ppb-month, no difference in FEV_1 due to TDI exposure compared to control values was observed, and no trend in FEV_1 decline with increasing cumulative TDI exposure was observed. The average annual FEV_1 decline for all workers was 30 ml/yr. The authors concluded that the decline in FEV_1 was within normal limits of age-related decline. Significant declines in FEV_1 were found among current smokers (1 ml/pack-yrs), those workers with asthma (114 ml/yr), and those with symptoms of shortness of breath (77 ml/yr).

A drawback of this study was that no short-term estimates of high TDI exposures were examined by the authors. Also, the health surveys used in this study could not adequately differentiate between occupational and nonoccupational asthma, although no workers were reportedly transferred due to TDI sensitization over the previous 10 years. The prevalence of asthma, and clinical symptoms including persistent cough and shortness of breath were not different in TDI workers when compared to the control group. Finally, pulmonary examinations of those that had left TDI operations had similar FEV_1 values as those control workers that left the hydrocarbons departments (-32 ml/yr). Thus, no negative impact on respiratory function was apparent after a worker had left the TDI operations.

Gui et al., 2014

A group of 49 workers were evaluated for respiratory health through the first year of employment at a new polyurethane production plant in Romania that installed state-of-the-art technology in foam production (Gui et al., 2014). The workers were divided into three exposure groups depending on their primary work location and duties. Workers producing foam in the foaming hall were considered to be the high exposure group; workers in the foam cutting area, laboratory, and in maintenance were in the medium exposure group; administrative, quality and warehouse workers were in the low exposure group. Airborne TDI levels were monitored by continuous fixed-point air sampling and limited personal monitoring of 7 workers in high and medium exposure groups. Some qualitative surface testing was conducted to evaluate sources of skin exposure.

Over the 1 year study period, 95 and 87% of the air recordings in the foaming hall and cutting area, respectively, were below the limit of detection of 0.1 ppb. The maximum recorded TDI vapor concentration was 10.0 ppb in the foaming hall and 5.4 ppb in the cutting area, and no air sampling period exceeded the ACGIH 8-hr TLV of 5 ppb. The authors note that TDI vapor levels from 0.5 to 5 ppb were commonly measured at two monitors in the foaming hall during peak foam production hours (10AM-2PM), and could have been higher at the source. All 7 personal monitors showed TDI levels below the limit of detection. Three of 11 SWYPE^{TM} samples were positive and the hands of one worker were positive for TDI exposure. Thirteen workers reported potential skin contact on the questionnaire.

Twelve of the initial 49 workers were lost to follow-up at 12 months. The reasons workers had resigned from the company or refused to participate were not available. However, the prevalence of current asthma was significantly higher in the workers lost to follow-up compared to those who completed the 12-month follow-up (25% vs. 2.7%, p=0.04). Three workers (7.1%) reported new asthma-like symptoms during the study, including one cutting area worker who reported a temporal relationship of his symptoms with work at 6 months, and by 12 months had resigned and left the workplace. Serum TDI-specific IgG results showed only one worker in the laboratory with elevated TDI-specific IgG at 6 months, but not at 12 months following transfer to a low exposure job. No elevated TDI-specific IgE was observed in any of the workers at 6 months (IgE test was not conducted at 12 months).

For spirometry testing, logistical issues resulted in only 24 of 49 workers being tested at baseline. There were no significant differences in FEV_1, FVC and FEV_1/FVC comparing baseline, follow-up and the workers lost to follow-up, or when comparing only those workers with spirometry data at all three time points (n=16), although the investigators reported a trend towards lower FEV_1 and FVC values at baseline vs. follow-up in this subgroup (data not shown). At 12 months, one worker showed new airflow obstruction (FEV_1/FVC < lower limit of normal) and three workers showed a decline in $FEV_1 \geq 15\%$, one of whom reported new asthma symptoms. Of note, three other workers exhibited an increase in $FEV_1 \geq 15\%$ at 12 months.

No significant associations in asthma-like symptoms and spirometry findings were observed between the three exposure risk groups. The small sample size and low prevalence of symptoms and other outcomes limited the statistical power to compare workers in the different exposure groups. However, the authors also noted greater potential skin and inhalation exposure to TDI occurred in the medium exposure group where glove and respirator use was limited. Overall, the authors concluded that recurrent low-level exposure to TDI occurs even in state-of-the-art facilities designed to limit exposure, that first year follow-up showed seven workers developed respiratory symptoms, and that TDI-specific IgG and/or changes in spirometry could represent early TDI-related health effects.

Table 14. Summary of Longitudinal Studies of TDI-exposed Workers

Study	Industry type & Exposure	Incidence of Sensitization	Pulmonary Findings
Weill et al., 1981; Diem et al., 1982	TDI manufacturing 5 yr study 277 male workers Mean adjusted age 35.6 yrs 8-hr TWA: 0.9 & 1.9 ppb for never-smoker subgroups	12/277 (4.3%) 0.9%/yr for workers identified as clinically "sensitive"	Mean FEV_1 decline of 38 ml/yr greater in high (n=21) vs. low (n=35) exposure never-smokers (p<0.001) No FEV_1 annual loss when cumulative dose treated as a continuous variable
Peters and coworkers	Polyurethane plant 2 yr study 38 workers initially Mean age 36.3 yrs Area sampling, 9-12 ppb in pouring area, 4-5 ppb near stripping molds	None found, but workers that left were not examined	Mean FEV_1 loss 110 ml/yr (p<0.01) after 2 yrs in 20 remaining workers; FEV_1 loss greater in workers with respiratory symptoms (probably chronic bronchitis) No control worker group
Wegman and coworkers	Polyurethane plant 4 yr study 63 workers initially Mean age 30.9 yrs Area and personal sampling; ≤2.0, 2.0-3.4, & ≥3.5 ppb groups	ND*, but sensitized workers likely included in analysis	Mean FEV_1 loss after 4 yr exposure of remaining 37 workers: 60 ml/yr in ≥ 3.5 ppb group 33 ml/yr in 2.0-3.4 ppb group No change in ≤ 2.0 ppb group (ANOVA, p=0.007) No unexposed control group
Musk et al., 1982	Polyurethane plant 5-yr study 107 workers Age not indicated 20-60 min sampling at peak exposure Mean 1.0-1.5 ppb (90^{th} %tile - 5 ppb)	No indication of sensitized workers	Mean FEV_1 loss after 5 yr exposure: 26 ml/yr in TDI workers (n=17) 22 ml/yr in controls (n=42) FEV_1 loss significantly related only to smoking
Omae, 1984	TDI manufacturing 2-yr study 145 workers initially Mean age 36.6 yrs (TDI workers); 37.9 yrs (referents) Personal monitoring 8-hr TWA 0.7-1.0 ppb	8/106 (7.5%) sensitization probably prior to study	Mean FEV_1 loss after 2 yr exposure: 50 ml/yr in TDI workers (n=64) 65 ml/yr in controls (n=21) No statistically significant differences in FEV_1 loss

Study	Industry type & Exposure	Incidence of Sensitization	Pulmonary Findings
Omae et al., 1992	Polyurethane plant 4-yr study 57 exposed workers 24 referents Mean age range: 34.3 to 39.8 yrs 8-hr TWA personal monitoring: 0.1, 1.7 and 8.2 ppb and control groups	ND, may have only examined "survivor" population	No average annual change in FEV_1 over 4 yrs for all groups. Lower MMF, MEF_{25}, and FEV% for high (n=15) vs. low (n=28) exposure ($p<0.05$); lower PEF for high vs. control (n=24) ($p<0.05$)
Jones et al., 1992	Polyurethane plant 5-yr study 380 workers initially Mean age: 37.3 yrs 8-hr TWA: 1.2 to 4.5 ppb based on job 9% of samples >5 ppb overall and 20% of samples ≥5 ppb in polyurethane production jobs	12/262 (4.6%) 0.9%/yr	Marginal effect on FEF_{25-75} ($p<0.063$) when cumulative TDI exposure treated as a continuous variable. Average annual FEV_1 loss (group n not provided): 53 ml/yr in never smokers 59 ml/yr in previous smokers 67 ml/yr in current smokers Declines steep in relation to expected values but not related to TDI exposure
Clark et al., 1998	Polyurethane plants 5-yr study 780 workers initially Mean age range: 35.5-39.2 yrs 8-hr TWA: 0.3 ppb control group 0.6 ppb low group 1.2 ppb high group 4.7% of samples exceeded 5.8 ppb	24/780 (3.1%) 0.6%/yr At least 14 sensitized were from the high exposure group	Average annual FEV_1 loss over 5 yrs: 38 ml/yr in high exposed group (n=521); not different from control (n=136) or low exposed (n=123) groups Mean daily excess decline in FEV_1 ($p=0.016$) in early exposure years of naïve workers
Clark et al., 2003	17-yr follow-up of Clark et al. (1998) workers 251 workers remain Mean age 48 yrs 1.05 ppb for high group: 1.3% of samples exceeded 5.8 ppb No exposure data for other 2 groups	ND; only examined survivor population	High exposed group (n=175): No relationship between the annual loss of FEV_1 (35 ml/yr) and FVC (30 ml/yr), and the mean daily TDI exposure. Excess declines in FEV_1 and FVC for low exposure group (n=26, data not shown in study) compared to the control subjects (n=50), but n low and higher proportion of smokers.

Study	Industry type & Exposure	Incidence of Sensitization	Pulmonary Findings
Ott et al., 2000	TDI manufacturing 5-yr retrospective analysis 313 exposed workers 158 referents Mean age of groups only stated as similar Ave 8-hr TWA 1976-88: 5.9 ppb 1989-97: 2.8 ppb	20/313 (6.4%) (1.1%/yr) incidence 1.1%/yr <1980; incidence 0.7%/yr >1979 Likely analysis of "survivor" population	Mean exposure duration 9.3 yrs. Average annual FEV_1 loss: 37 ml/yr all workers (n=209) 35 ml/yr controls (n=65) 31 ml/yr never smoker workers (n=67) 36 ml/yr never smoker controls (n=25) Annual FEV_1 loss not associated with exposure
Bodner et al., 2001	TDI manufacturing 305 TDI workers: 8-hr TWA 2.3 ppb (96.9 ppb-month) 3.8 yrs mean employment and mean age of 34.4 yrs 581 control workers	No difference from controls, but did not differentiate between occupational and nonoccupational asthma	No difference in FEV_1 among cumulative exposure groups of 1-12,13-60, 61-120 and >120 ppb-mo No FEV_1 change compared to controls No trend in FEV_1 decline with increasing cumulative TDI exposure
Gui et al., 2014	TDI polyurethane plant 1st yr of study 49 workers Mean age 39.1 yrs 0.5-5 ppb near foam production area; below detection limit of 0.1 ppb 87-95% of the time	3 workers developed new asthma symptoms but sensitization not confirmed	7 workers developed either new asthma symptoms (n=3), TDI-specific IgG (n=1), new airflow obstruction (n=1) and/or a decline in $FEV_1 \geq 15\%$. No differences found in health outcomes when stratified into 3 exposure groups (high (n=13), medium (n=28), low (n=8)), but sample size too small.

* ND – Not determined

6.1.6. Additional supporting studies

Surprisingly few of the longitudinal studies summarized above gave strong evidence of a quantitative exposure-response relationship for TDI-induced asthma. The following case-referent and cross-sectional studies provide the best available data for exposure levels to TDI that can result in development of occupational asthma. A few studies also provide evidence for decreased lung function in non-sensitized workers with long-term exposure.

Lee and Phoon, 1992

This cross-sectional occupational study presents evidence of pulmonary function decrements with prolonged high occupational exposures to TDI (Lee and Phoon, 1992). Pulmonary effects of TDI exposure were studied in 26 mixers from eight east Asian factories making polyurethane foam, and in 26 unexposed controls

matched for age, race and smoking. Mean exposure duration for the TDI workers was 6.6 yrs. Personal breathing zone samples (n=24) were collected only during the foaming process when TDI exposure was the highest, a duration of 30 to 92 minutes. Analysis was by the Marcali method, so only mean exposure time could be determined and the exposure may be underestimated. The foaming process was carried out for 4 to 6 hours every day at 6 factories and for only about 4 hours per week at two factories that had automated the process.

The mean TDI exposure concentration during the mixing process was 160 ppb (range: 10 to 500 ppb). Prevalence of symptoms included increased eye irritation and cough in the mixers compared to unexposed controls. Only one case of clinically overt wheezing was observed in exposed workers, and this subject was negative when tested by bronchial challenge to TDI. The authors characterized the group of mixers as a survivor population in which only those workers that did not develop TDI-related asthma remained in the job.

No statistically significant differences were observed in FEV_1 or FVC between the TDI workers and the control group. FEV_1 of mixers was 3.4 L, and that of the controls was 3.5 L. There was a statistically significant reduction (p=0.01) in the average ratio of FEV_1/FVC and in diurnal variation of peak expiratory flow (PEF) rate (p=0.02). Six mixers exhibited a diurnal variation in PEF of more than 15% on at least one day compared with none among the controls (p=0.01; Fisher's exact test).

Mixers with exposure of 10 years or more (n=7) had a significantly reduced FEV_1 (2.7 L; $p<0.001$) compared to those with less than 10 years of exposure (FEV_1=3.5 L, n=19) and controls (FEV_1=3.5 L, n=26). FEV_1 was reduced 16.4% in this group compared to predicted values. Workers with >10 years of exposure were older (39.0 yrs) than workers with <10 years of exposure (28.8 yrs) and the controls (32.2 yrs), but FEV_1 had been adjusted for age, height, race, and cigarette-yrs. The mixers with >10 years of exposure also exhibited a reduced FVC ($p<0.05$ compared to mixers with <10 yrs of exposure only), FEV_1/FVC ($p<0.005$ compared to control group only), and diurnal variation in PEF ($p<0.05$ compared to controls only). The authors suggested that the longer TDI exposures were associated with chronic airways obstruction.

Huang et al., 1991

This cross-sectional study from China compared the pulmonary function of 15 painters (7 men and 8 women) exposed to high levels of TDI from polyurethane varnish with 18 referents with no history of TDI exposure (Huang et al., 1991). The painters had a mean exposure duration of 7.5 yrs to TDI. Referents (9 men and 9 women) were matched with regard to age, height, weight and smoking habits. Area sampling with analysis by a colorimetric method on the day of pulmonary testing revealed TDI concentrations of 70-170 ppb during the 8-hr workshift.

Respiratory symptoms included eye, nose and throat irritation in all exposed painters. Seven painters with chronic bronchitis, and four with longer exposure durations (9.5-17 yrs) reported dyspnea and wheezing during work. Five painters had a positive response with patch testing to 0.1%TDI in petrolatum. All symptoms were statistically significantly different compared to referents ($p<0.01$).

Pulmonary function testing revealed reduced FEV_1 in the painters compared to referents (2.04 L vs. 2.94 L, $p< 0.05$). The $\%FEV_1$ and maximum mid-expiratory flow were also statistically significantly reduced in the exposed workers. The four painters exhibiting wheeze and dyspnea during work, reported by the authors to be consistent with work-related asthma, had a much greater rate of decline than the other painters (data not shown).

Meredith et al., 2000

The quantitative relationship between exposure to diisocyanates and occupational asthma was investigated at several polyurethane plants (Meredith et al., 2000). Workers with diisocyanate-induced asthma (n=27; 24 exposed to TDI and 3 exposed to MDI) were compared with referents (n=51) that were defined as workers without asthma, of the same sex as the case, who were working in the same area, both at the time the case started the job in which he developed asthma and when the diagnosis of occupational asthma was made. Exposures by job category were reconstructed based on records of personal paper-tape monitors.

No difference was found between the 27 cases and 51 matched referents in estimated peak exposure (means 21.5 ppb and 22.5 ppb respectively), defined as the highest 15-20 min TWA exposure. Mean 8-hr TWA exposure for cases (1.5 ppb) was slightly higher than for referents (1.2 ppb). The odds of occupational asthma for those for whom estimated exposure to TDI was greater than the median concentration for the control group (1.125 ppb) were 3.2 times the odds for those exposed to lower concentrations (95% confidence interval 0.96 to 10.6; $p=0.06$). The odds of occupational asthma increased by 8% for each 0.1 ppb increase in 8-hr TWA exposure ($p=0.06$). Overall, higher exposures seemed to be associated with increased risk of disease, but this was limited to those cases which occurred in the first year of employment. A history of either hay fever, eczema, or asthma at the time of employment tripled the risk of developing occupational asthma ($p=0.04$), but no single factor was associated with significantly increased risk.

This study suggests that keeping 8-hr TWA exposure below 1 ppb significantly lowers the risk of TDI-induced asthma in a worker population. A limitation of this study was that person-specific exposure measurements were not available, and so exposure was estimated from job title and date. Referents were closely matched to cases and selected from the same work areas in an attempt to ensure a similar level of surveillance and a similar chance that occupational

asthma would be diagnosed if it occurred. This was done, the authors noted, to minimize the risk of selection bias, a common problem in case-control studies.

Tarlo et al., 1997

In this study conducted in Ontario, Canada, the ambient levels of isocyanates were compared between 20 plants with and 203 plants without cases of compensated isocyanate occupational asthma over a 4-year period from 1984-1988 (Tarlo et al., 1997). Sampling methods included Marcali and Nitro reagent methods. Exposure was based on the highest level identified at a plant as a result of state-mandated monitoring at plants to ensure worker exposure does not exceed 5 ppb. There were a total of 49 occupational asthma claimants in which exposure was attributed primarily or exclusively to TDI. Some claimants also had potential exposure to MDI and/or HDI. The overall estimated incidence of occupational asthma in the total 223 companies surveyed was 0.9% in the 4 years of the study (56 out of 6,308 workers).

A greater proportion of companies which had claims for occupational asthma was found in the higher exposure category (≥5 ppb) than in the lower exposure category (<5 ppb). For TDI, the proportion was 30% (high) vs. 13.7% (low); the odds ratio (OR) was 2.7 (95% confidence interval (CI) of 0.7-10.6). When combined across all isocyanate types, claims were significantly more likely to be from companies in the higher exposure category; 10 of 20 companies (50%) with claims were in the high exposure category vs. 50 of 203 companies (25%) without claims, OR: 3.1 (95% CI: 1.1-8.5, two-tailed p=0.03).

6.2. Chronic Toxicity to Infants and Children

No studies of inhalation exposures to TDI among children were located. It has been postulated that early life exposure to TDI may occur through inhalation and dermal contact with polyurethane products (Krone et al., 2003). Strachan and Carey (1995) found independent associations between severe wheeze and the use of non-feather bedding, especially foam pillows (odds ratio 2.78; 95% C.I. 1.89 to 4.17), among children with 12 or more wheezing attacks in the previous 12 months. The authors speculated that volatile organic compounds could be off-gassing from the foam pillows. However, other researchers found that there is increased exposure to house dust-mite allergen from synthetic pillows compared to feather pillows and that this may explain the increased asthma symptoms (Crane et al., 1997).

In addition, three studies did not find emissions of detectable levels of free TDI from polyurethane foam or consumer products that were made with TDI (e.g., carpet padding, mattress and furniture foam, varnishes and sealants) (CARB, 1996; Hugo et al., 2000; Vangronsveld et al., 2013b). Krone et al. (2003) applied semiquantitative tests (i.e., wipe test and extraction with dimethyl sulfoxide) for isocyanate to polyurethane products, including mattresses, mattress pads, sofa padding, carpet pads and pillows, and detected free

isocyanate in these consumer products. It was suggested that isocyanate may be available to dissolve in skin oils upon dermal contact. A similar study by Vangronsveld et al. (2013a) used various solvent systems and detection methods to extract free TDI from flexible polyurethane foam. A toluene-based extraction technique was deemed the most consistent and resulted in microgram per gram levels of free TDI extracted from the foam. The authors hypothesized that the TDI extracted from foam may have been due to decomposition of parts of the foam structure by the solvent, a process that is unlikely to occur under typical household uses.

Vangronsveld et al. (2013b) measured the amount of solvent-extractable (unreacted) 2,4- and 2,6-TDI in polyurethane foam and attempted to more accurately estimate consumer inhalation and dermal exposures to TDI from normal foam use. Using a Field and Laboratory Emission Cell (FLEC) and a Micro-Chamber/Thermal Extractor™, a clean airstream was passed over the surface of the polyurethane foam producing a TDI emission which then passed through a sampling train. No emitted TDI was detected at the lowest method detection limit (0.002-0.003 ng/m^2). For estimating dermal exposures resulting from skin contact with a polyurethane foam mattress without sheets/pads, TDI migration out of the foam was measured by sandwiching and compressing it between eight stacked glass fiber filters impregnated with 1-(2-methoxyphenyl)-piperazine, a derivatizing agent. Migration sampling was done over a period of eight hours. Solvent-extracted foam representative of those used for the emission and migration tests yielded 56 ng and 240-2800 ng TDI, respectively. However, inhalation and dermal tests simulating human exposure to foam resulted in negligible exposure to TDI, less than the limit of detection for the various methods (<1 ng total, <1 ng/g foam, and <1 ng/cm^2 foam). OEHHA notes that Vangronsveld et al. (2013b) relied on filter-based emission sampling, which can miss a significant fraction of TDI relative to filter sampling combined with an impinger, and did not test the chamber walls for adsorbed TDI.

It is unknown how the immune system in infants and children would respond to TDI exposure during critical stages of immune system and respiratory system development. At birth, humans exhibit a dominant humoral, T_H2, responsiveness (i.e., an atopic state). During the first few years of life, the T_H2 response converts to a more cellular (T_H1) immune response characteristic of the mature adult immune system. A delay in the transition from the predominant T_H2 pattern to the more balanced T_H1/ T_H2 response allows an atopic T_H2 type response to persist longer, thus extending the period of vulnerability to environmental stressors and allergens, and increasing the likelihood of subsequent disease expression including asthma (Prescott et al., 1999; Wills-Karp, 1999). Contrary to a T_H2 pattern for childhood atopic asthma, obese children with asthma exhibit T_H1 polarization and greater asthma severity, whereas lean children with asthma exhibit T_H2 polarization and less asthma severity (Youssef et al., 2013). The presence of high leptin levels in the obese children is associated with an increase in IFN-y production by T_H1-polarized cells. Leptin is found in higher levels in obese children and is known to promote the production of nitric oxide and pro-

inflammatory cytokines in macrophages and monocytes. So, depending on body weight of the child, this research suggests either T_H1- or T_H2-driven pathways can be involved in childhood asthma.

While there is evidence that atopic asthma in children is usually T_H2-based, the immunopathogenesis of diisocyanate-induced asthma is less distinct. Diisocyanate-induced asthma in workers has shown either a T_H1 immune response pattern (Finotto et al., 1991; Maestrelli et al., 1994) or a mixed T_H1/ T_H2 immune response (Maestrelli et al., 1997; Redlich et al., 1997; Lummus et al., 1998). Regardless of the differences in T cell profiles, the clinical manifestations and pathophysiological changes observed in TDI-induced asthma are remarkably similar in some respects 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).

Similar to development of childhood allergic asthma, diisocyanate-induced asthma is multifactorial in origin and complex. The mechanism of sensitization by diisocyanates is not well understood in adults, much less children. Thus, differences in T cell profiles in childhood atopic asthma and diisocyanate-induced asthma does not inform us regarding the response of immune systems in infants and children to TDI exposure.

6.3. Chronic Toxicity to Experimental Animals

One lifetime TDI exposure study in rodents has been carried out and reported by Loeser (1983). Groups of male and female rats and mice (~40 rats and 30 mice per sex per level) were exposed to 0.05 and 0.15 ppm TDI by whole-body inhalation for 6 hr/day, 5 days/week for approximately 2 years. In rats, termination of the study occurred at week 110 for males and week 108 for females. TDI exposure did not affect mortality, although mortality was high in all groups (64 to 75%). Male and female rats in the 0.15 ppm group gained less weight, but only during the first 12 weeks of exposure. No treatment-related changes in hematological, blood biochemical or urinary parameters were seen. Organ weights were not affected by TDI exposure and no macroscopic changes were observed in the upper respiratory tract. The methodology section noted that histopathologic examination of infused lungs occurred but the findings were not reported.

Histopathology of the rat nasal turbinates in the Loeser (1983) report were reported separately by Owen (1984). Seven rats per sex per level were examined at 6-, 12- and 18-month interval sacrifices, respectively. There were 26 to 37 rats per sex per level sacrificed and examined at termination of the study at approximately 2 years. At 6-, 12- and 18-month sacrifice, nasal passages showed a dose-related increased incidence of rhinitis mainly in the anterior portion of the nasal cavity (Table 15). The lesions at 6-, 12- and 18-months showed similar incidences and severity grades regardless of exposure duration, so the data were combined in Table 15. Rhinitis was

generally characterized by squamous metaplasia/hyperplasia of the respiratory mucosa, with or without exudates in the lumen, and leukocyte infiltration. In males, there was some degree of rhinitis in about half of the controls. Female control rats were free of rhinitis.

Table 15. Summary of Incidence of Grade Scores in the Anterior Respiratory Portion of the Nasal Cavity of Rats Exposed to TDI: Six-, 12- and 18-Month Sacrifice Combined

Histopathology Score[a]	TDI Exposure Group (ppm)					
	Male 0	Male 0.05	Male 0.15	Female 0	Female 0.05	Female 0.15
# examined	21	20	21	21	21	20
Grade 0	13	12	2	21	12	7
1	3	2	0	0	4	6
2	4	4	5	0	5	5
3	1	2	11	0	0	2
4	0	0	3	0	0	0
% with grade 3-4	5	10	67	0	0	10

[a] Histopathological grading scores: (0) unremarkable, (1) minimal - rhinitis present in <25% of mucosa, (2) slight – rhinitis present in 25-50% of mucosa, (3) moderate – rhinitis present in 50-75% of mucosa, and (4) marked – rhinitis present in >75% of mucosa.

At 2-year sacrifice, the dose-related increased incidence of rhinitis was still apparent, although some female control rats now showed some degree of rhinitis. In particular, the incidence and severity grade of the lesion had increased in exposed females at two years more than in exposed females in the earlier sacrifice groups (Table 16). To a lesser extent, nasal lesions were also found in the middle and posterior nasal cavity. Overall, Owen (1984) concluded that the nasal lesions were a low grade injury considered to be evidence of local irritation. The lesion was not accompanied by any unusual proliferative changes suggestive of any oncogenic effect.

Table 16. Summary of Incidence of Grade Scores in the Anterior Respiratory Portion of the Nasal Cavity of Rats Exposed to TDI: 2-Year Sacrifice

Histopathology Score[a]	TDI Exposure Group (ppm)					
	Male 0	Male 0.05	Male 0.15	Female 0	Female 0.05	Female 0.15
# examined	37	35	30	35	26	36
Grade 0	17	18	4	25	15	10
1	8	5	5	7	4	10
2	9	11	8	3	4	13
3	3	1	12	0	3	3
4	0	0	1	0	0	0
% with grade 3-4	8	3	43	0	12	44

[a] Histopathological grading scores: (0) unremarkable, (1) minimal - rhinitis present in <25% of mucosa, (2) slight – rhinitis present in 25-50% of mucosa, (3) moderate – rhinitis present in 50-75% of mucosa, and (4) marked – rhinitis present in >75% of mucosa.

In mice, Loeser (1983) observed a statistically significant increase in mortality in females of both dose groups, although not strictly dose-related (60% in controls, 77% low-dose, 74% high-dose). The female mice in the 0.15 ppm group showed a high incidence of death within a 3 week period during the 10th month. Rhinitis in this group was considered to be associated with exposure and was predominantly seen in the animals dying during the study. Statistically significant reduced weight gain was observed in the high dose group for both males and females. No treatment-related changes in hematological, blood biochemical or urinary parameters were seen. The major pathological change observed in both groups was a dose-related increased incidence and severity of either chronic or necrotic rhinitis (epithelial atrophy, mucous and squamous metaplasia, inflammation, focal destructive rhinitis with debris). Lesions of variable incidence and severity were also seen in the lower respiratory tract (interstitial pneumonitis, catarrhal bronchitis) and in the eyes (keratitis) of some mice, with a higher incidence in the 0.15 ppm group. Pathology tables for the mice, including specific incidence and severity grades for respiratory tract findings, were not provided in the report.

Human occupational studies show that TDI exposures in the ppb range result in adverse effects. Thus, only the principal subchronic animal exposure studies (i.e., <2 year exposure in rodent models) that conducted toxicity studies at TDI concentrations in the range of high occupational exposures (5-20 ppb) are summarized.

Animal models for TDI-induced allergic rhinitis and asthma have been developed and suggest a duration-dependent nature of the response. Female C57BL/6 mice were given a subchronic nose-only exposure to TDI vapors (80:20 2,4:2,6

isomers) at 20 ppb, 4 hr/day, 5 d/wk for 6 weeks, or a single acute, 2 hr exposure to 500 ppb (Matheson et al., 2005). These exposures were followed 14 days later with an acute 1 hr challenge exposure to 20 ppb. Three control groups included air-exposed/air-challenged, TDI-exposed/air-challenged, and air-exposed/TDI-challenged mice. A portion of each group was euthanized 24 hr after the challenge for the collection of blood and bronchioalveolar lavage fluid (BALF). Another portion was euthanized 48 hrs after challenge for airway and pulmonary histopathology. The exposure period resulted in different immunological response patterns. Following the challenge exposure, total IgE levels were elevated 10-fold over controls ($p < 0.05$) in mice with the subchronic exposure but not significantly different from controls in acutely exposed mice. However, both subchronic and acute exposures significantly raised TDI-specific IgG antibodies ($p < 0.05$) compared to non-detectable levels in controls. Subchronically exposed mice exhibited histopathological changes in the nares and lungs consistent with an inflammatory response including significant infiltration by neutrophils, lymphocytes, eosinophils, and macrophages.

In acutely exposed mice, Matheson et al. (2005) observed that inflammatory cells were only slightly elevated relative to controls. Consistent with this, inflammatory cytokines (IL-4, IL-5, IFNγ and TNFα) were significantly ($p < 0.05$) elevated following subchronic but not acute exposure. However, both exposure regimens resulted in significant degenerative cellular changes including loss of cilia, goblet cell metaplasia, septal exudates, hyaline droplet formation and epithelial hyperplasia. In addition, airway hyperreactivity, assessed by methacholine challenge, was significantly elevated in animals sensitized to TDI and challenged with TDI regardless of exposure duration. These results suggest that long term, low level TDI exposure caused a marked allergic response including airway inflammation, eosinophilia, goblet cell metaplasia, elevated IgE and TDI-specific IgG, and T_H1/ T_H2 cytokine expression, all characteristic of allergic asthma. Acute exposure however, did not present evidence of either T_H2 or eosinophil involvement, and the observed cellular changes likely were predominantly a result of upper airway irritation rather than an immune response.

To examine the effects of long-term, low-level exposure on bronchial hyper-responsiveness, English smooth-haired guinea pigs were exposed to 0.02 ppm TDI in an inhalation chamber 6 hr/day for 70 days (Karol, 1983). Other groups of guinea pigs were exposed to 0.12 to 10 ppm TDI for 3 hr/day for 5 days. Animals receiving short term exposures to 0.36 ppm and above displayed bronchial hyper-responsiveness and antibody production. None of the animals with short-term exposure to 0.12 ppm TDI or low level (0.02 ppm) subchronic TDI exposure developed antibodies or pulmonary responsiveness upon challenge. Animals exposed to 2 ppm and above in the short term exposure experiment displayed pneumotoxic responses and few pulmonary hypersensitivity reactions. These results suggest that in the absence of a sensitizing event, chronic low level TDI exposure may not elicit either pulmonary hyper-responsiveness or immune responses.

Pulmonary function was investigated in guinea pigs exposed head-only to 0 or 20 ppb TDI, 6 hr/day, 4 days/wk for 14 weeks (Wong et al., 1985). Each week during exposure, eight animals were randomly selected and placed in a whole-body plethysmograph to record respiratory frequency, pressure change (which is proportional to tidal volume) and the ventilatory response to 10% CO_2. These animals were compared to guinea pigs receiving 1.4 ppm TDI 3 hr/day for 4 days, a concentration expected to cause pulmonary damage. Exposure to 20 ppb TDI had no effect on baseline pressure change, respiratory frequency or the ventilator response to CO_2, whereas exposure to 1.4 ppm TDI increased baseline pressure change and respiratory frequency, but diminished the CO_2-induced increase in pressure change. All animals exposed to 1.4 ppm showed multifocal subchronic interstitial inflammation at terminal sacrifice, whereas only 2 of 24 animals exposed to 20 ppb TDI showed patchy interstitial inflammation.

Bronchial provocation challenge was also examined by Wong et al. (1985) with TDI antigens on days 37-38. Four of 8 guinea pigs exposed to 1.4 ppm TDI exhibited pulmonary hypersensitivity. No sensitivity was apparent in animals exposed to 20 ppb TDI or control animals.

Immunologic and respiratory responses have been produced in dogs with airway challenge to TDI (Patterson et al., 1983). In this model three anesthetized dogs received 1 mg/kg aerosolized TDI via tracheal tube every 2 weeks for 4 months, and then every 4 weeks thereafter for 6 months. A dose of 1 mg/kg TDI every 4 weeks was said to approximate 4 week exposure of a human at heavy work exposed to 20 ppb TDI. After the biweekly exposures, the dogs developed systemic immune responses to TDI-dog serum albumin, which included IgG, IgA and IgM antibody responses and development of lymphocyte reactivity. Immediate-type airway responses occurred after the fourth TDI aerosolization, some of which qualitatively simulated IgE-mediated antigen-induced airway responses.

As first discussed with Acute Toxicity, Section 5.3, a review of animal models for the adverse effects of TDI by Schupp and Collins (2012) concluded that respiratory irritation and sensitization may be interdependent, and both irritation and sensitization by TDI are threshold phenomena. Animal species investigated and summarized in this review were primarily guinea pigs and mice, but included a few studies in rats and one in rabbits. Multiple factors affect the thresholds, including diisocyanate potency, route of exposure, the extent, duration and frequency of exposure as well as other factors including genetic susceptibility and other underlying disease conditions. Nevertheless, the majority of the animal NOAELs for respiratory sensitization were in the range of 5 to 30 ppb, whereas the LOAELs were about 20 to 400 ppb. The threshold for respiratory sensitization appears to hold regardless of whether exposure duration was acute (i.e., two weeks or less) or subchronic in length, as long as the $C \times t$ paradigm results in a dose that does not overcome the scrubbing ability of the upper airways.

6.4. Toxicogenomics

Even though diisocyanates are one of the most common causes of occupational asthma, only 5 to15% of exposed workers develop the disease. Thus, genetic variability has been implicated in the susceptibility to occupational asthma by TDI and other diisocyanates. A number of gene variants have been reported to be associated with increased sensitivity to the disease in workers (Table 17), which suggests that diisocyanate-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 in the workplace. Since exposure to the other major commercial diisocyanates, MDI and HDI, result in sensitization and asthma in susceptible individuals similar to TDI, the combined toxicogenomic findings for all three are presented here for a more complete picture of the influence of genotype on the health effects of diisocyanates.

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 diisocyanate-induced asthma (DA). The study population consisted of 353 diisocyanate (TDI, MDI, and HDI) exposed Caucasian French-Canadian workers recruited from occupational clinics in Canada or, in the case of asymptomatic workers, from painters in Quebec, Canada exposed to HDI. The workers were divided into three groups: 95 workers with specific inhalation challenge confirmed DA (DA+); 116 symptomatic diisocyanate workers with a negative specific inhalation challenge (DA-); and 142 asymptomatic exposed workers (AW). Specific inhalation challenge with the work-related diisocyanate resulting in a 20% drop in FEV_1 was considered positive for DA. The investigators analyzed the role of gene variants for antioxidant defense genes previously shown to modulate susceptibility to asthma and other inflammatory respiratory 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 17 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 17). 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 contribute 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.

Piirila et al (2001) evaluated polymorphisms in glutathione-S-transferase genes (GSTM1, GSTM3, GSTT1, and GSTP1) to look for associations with 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, atopy (Table 17).

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. Mapp et al. (2000) 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 also 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. These data suggest that genotype for HLA class II molecules influences risk of toluene diisocyanate-induced asthma.

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). 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 were examined to determine if genetic variants of CTNNA3 genes are associated with increased susceptibility to DA. 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 7088181 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. These same SNP CTNNA3 polymorphisms were also significantly associated with TDI-induced asthma in a group of 84 Korean workers compared to 263 normal controls (Kim et al., 2009).

Sixty-two workers with DA and 75 diisocyanate workers negative for DA were analyzed for SNPs associated with the immune response genes IL4RA, IL-13,

and CD14 (Bernstein et al., 2006). The T_H2 cytokines IL-4 and IL-13 play key roles in airway inflammation and allergic disease and SNPs of both the IL-13 and the IL4 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.

Table 17. Variability in Observed Odds Ratio (OR) or *p* Value for Significant Genotype Variation Associations and Increased Susceptibility for Diisocyanate-Induced Asthma

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

Reference	Odds Ratio and/or *p* value	Genetic associations for DA
Mapp et al., 2000	*P*=0.005	**HLA DQA1*0104** carried by 16 of 67 cases TDI-induced asthma (23.9%), 0 of 27 asymptomatics (0%)
	P=0.009	**HLA DQB1*0503** carried by 14 of 67 cases TDI-induced asthma (20.9%), 0 of 27 asymptomatics (0%)
	P=0.027	**HLA DQB1*0503** carried by 14 of 67 cases TDI-induced asthma (20.9%), 9 of 101 normals (8.9%)
Kim et al., 2006	*P*=0.001 (cases vs. asymptomatics) *P*=0.003 (cases vs. normals)	**HLA DRB1*15-DPB1*05** carried by 10.6% in cases (n=110), 0% in exposed asymptomatic worker controls (n=94), and 2.5% in unexposed normals (n=190).
Choi et al., 2009	TDI-OA vs. AEC[c] OR=4.43 (95%CI 1.50-13.10) *p*=0.007	**DRB1*1501-DQB1*0602-DPB1*0501** carried by 16 of 84 cases (19%), 1 of 47 asymptomatic workers (2.1%), and 4 of 127 normals (4%).
	TDI-OA vs. AEC OR=2.024 (95%CI 1.14-3.59) *p*=0.016	**DRB1*1501- DQB1*0602** carried by 23 of 84 cases (27.4%), 6 of 47 asymptomatic workers (12.8%), and 15 of 127 normals (11.8%).
	TDI-OA vs. AEC OR=3.127 (95%CI 1.38-7.08) *p*=0.006	**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%).
Bernstein et al., 2013	OR=9.05[d] (95%CI 1.69-48.54) *p*=0.01	**CTNNA3 (rs7088181)** – homozygous for SNP minor allele comparing DA+ vs AEC
	OR=6.82 (95%CI 1.82-14.88) *p*=0.002	**CTNNA3 (rs10762058)** – homozygous for SNP minor allele comparing DA+ vs AEC
Bernstein et al., 2006	OR=5.2 (95%CI 1.65-28.24) *p*=0.008	**IL4RA (150V) II and CD14 (C159T) CT** HDI workers with DA 39% vs 11% among DA-negative workers
	OR=6.4 (95%CI 1.57-26.12) *p*=0.01	**IL4RA (150V) II, IL13 (R110Q) RR, and CD14 (C159T) CT** HDI workers with DA 24% vs 5% among DA-negative workers

[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 diisocyante-asthma vs asymptomatic HDI-exposed controls

7. Toxicity Studies of Toluene Diisocyanate Prepolymers

There are very few peer-reviewed toxicity studies for TDI prepolymers, which is likely a reflection of their lower and more recent use commercially compared to monomeric TDI. However, what these studies show is that TDI monomer and prepolymers share many of the same pulmonary effects, including inducing sensitization and occupational asthma with repeated exposure. This suggests some commonality in the mechanisms of sensitization, possibly related to N=C=O binding carrier proteins (Bello et al., 2004). Because of the limited toxicity data on TDI prepolymers, more information is needed to determine if the TDI RELs based on the monomer forms is sufficient for the prepolymers, or if new RELs are needed for TDI prepolymers.

Vandenplas et al. (1992) observed occupational asthma in two workers caused by a TDI prepolymer, but not monomeric TDI. The workers developed progressively worsening asthma symptoms over several years applying a varnish during wood-roof maintenance. The varnish contained 31.5% TDI prepolymer and 2.5% TDI monomer. Specific inhalation-challenge tests with the TDI monomer did not elicit significant airway obstruction, whereas exposure to the varnish and to the purified TDI prepolymer induced late asthmatic reactions. The authors do not identify the specific type of prepolymer(s) the workers were exposed to, but note that the most commonly used TDI prepolymer is a TDI trimer (see Figure 1).

Pauluhn (2004) compared the relative acute pulmonary irritant potency of several polyisocyanates, including a TDI-based polyisocyanate and a HDI-TDI polyisocyanate mixture, in male Wistar rats following 6-hour exposures to the aerosols. Monomeric isophorone diisocyanate (IPDI) served as a semi-volatile reference compound known to cause airway irritation rather than pulmonary irritation. Polyisocyanate aerosols, on the other hand, have been shown to result in pulmonary irritation rather than airway irritation. Pulmonary irritation was assessed by changes in lung weight, and total protein and lactate dehydrogenase (LDH) in bronchoalveolar lavage fluid (BALF). Increased total protein in BALF has been shown to be among the most sensitive indicators for the acute irritant effects of polyisocyanate aerosols and is indicative of irritant-related, functional impairment of the blood/air barrier (Pauluhn, 2002; 2004). LDH in BALF was used as a marker of cytotoxicity.

Most of the polyisocyanate aerosols displayed similar concentration dependence and time course for the endpoints (Pauluhn, 2004). 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 aerosols containing TDI polyisocyanate, 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 in the range of 40 to $\geq$50 mg/m^3 (Table 18). A NOAEL for this endpoint could be established for the TDI polyisocyanate (43 mg/m^3), but the highest concentration used for the TDI-HDI polyisocyanate did not result in a

measurable increase in total protein. For comparison, other HDI- and MDI-based polyisocyanates are shown in Table 18 had irritant thresholds in the range of 2-4 mg/m^3. 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, IPDI displayed increasing total protein and LDH in BALF up to day 7 post-exposure characteristic of an isocyanate in which upper airway irritation predominates over pulmonary irritation. An apparent NOAEL of 8 mg/m^3 for pulmonary irritation was determined by the author for IPDI, although he noted that upper airway effects not measured in this study may substantially lower the NOAEL.

Table 18 NOAELs and LOAELs in mg/m^3 for Various Pulmonary Endpoints in Rats Exposed Acutely to TDI, HDI and MDI Polyisocyanate Aerosols (Pauluhn, 2004)

Test Substance	NOAEL Endpoints			Respiratory Tract Irritation	
	Lung Weights[a]	Protein[a]	LDH[a]	NOAEL[b]	LOAEL[b]
TDI-HDI polyisocyanate	>50	>50	>59	50.9	>51
TDI-based polyisocyanate	>52	43	>52	52.3	>52
HDI Isocyanurate	24	3.5	21	15.9	54
HDI-based polyisocyanate	27	4.1	20	3.1	18
Polymeric MDI	>20	1.4	>20	2.4	8.1

[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

8. Developmental and Reproductive Toxicity

To examine the developmental toxicity of exposure to TDI vapors, Tyl et al. (1999a) exposed mated female Sprague Dawley rats to 0, 0.02, 0.10, or 0.50 ppm (25 per dose) of an 80:20 mixture of 2,4- and 2,6-TDI for 6 hr/day on gestation days 6-15. Animals were terminated on gestational day 21 and examined for signs of fetal and maternal toxicity. At 0.50 ppm, signs of maternal toxicity included reduced feed consumption and reduced weight gain, audible breathing and, among some animals, red nasal discharge. Reduced weight gain was also observed at the lower doses but this was reportedly transient in nature. No maternal treatment-related lesions were observed at necropsy. In terms of developmental toxicity, there were no apparent treatment-related effects on the total number of corpora lutea, implants per litter, or percent live fetuses. Of 111 skeletal variations observed, the only treatment-related sign of fetal toxicity was poor ossification of cervical centrum 5 at 0.50 ppm. The reported LOAEL for maternal and fetal effects was thus 0.50 ppm, with a corresponding NOAEL of 0.10 ppm.

The potential reproductive effects of TDI exposure were investigated in a two-generation study, also by Tyl et al. (1999b). Sprague-Dawley rats (28/sex/group) were exposed beginning at 42 days of age to TDI vapors (80:20 mix of 2,4- and

2,6-TDI) for 6 hr/day, 5 days/wk for 10 weeks at 0, 0.02, 0.08, or 0.3 ppm. This was the parental or F0 generation. During the 3-week mating period, gestation and lactation, exposures were increased to 7 days/wk. F0 maternal rats were not exposed from gestational day 20 through postnatal day 4, but exposures resumed on postnatal day 5. Randomly selected weanlings (F1) were exposed as described above for 12 weeks prior to mating, during the 3-week mating period, and throughout gestation and lactation. Males of the F1 generation were terminated after delivery of the F2 generation; F1 females were terminated upon weaning of the F2 generation.

Among F0 parents, reproductive parameters were unaffected by TDI treatment. In the production of the F1 generation, no treatment-related effects were seen on gestation length, litter sizes, sex ratios, pup body weights or weight gain. In the F2 generation, a transient significant decrease in pup body weight gain was observed at the 0.08 and 0.30 ppm level. At these exposure levels, pup body weight/litter was also depressed (transiently in females, permanently in males). In the F0 and F1, but not F2 generations, rhinitis showed a dose-dependent incidence and severity. This study found no effects of TDI exposure on reproductive parameters in either the F1 or F2 generations.

9. Derivation of Reference Exposure Levels

Exposure to TDI could result in several adverse health effects depending on the level and duration of exposure. These effects include 1) acute sensory irritation and respiratory inflammation, 2) asthmatic episodes in acutely-exposed non-sensitized asthmatic subjects, 3) sensitization and induction of asthma in susceptible individuals with frequent repeated exposure, 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 affects resulting from exposure to TDI emissions. In addition, the RELs also consider potential exposure of those individuals previously sensitized to TDI through occupational exposure or some other source, but cannot unequivocally protect all sensitized individuals in the general population (see discussion below).

Strong supporting data in animal models, with some limited data in humans, show that prevention of a TDI inhalation dose that causes pulmonary irritation and inflammation will also deter the initiation of pulmonary sensitization (Vandenplas et al., 1993; Pauluhn, 2004). The scrubbing ability of peptides (i.e., GSH) and proteins in epithelial lining fluid of the upper airways will prevent TDI from reaching susceptible regions in the lower respiratory tract, provided the inhalation dose is low enough. Thus, the threshold for pulmonary irritation and sensitization are interrelated and based on the $C \times t$ relationship where the total dose is the best predictor of the threshold for penetration of TDI to the susceptible regions in the intrapulmonary region. The accelerated decrease in lung function (i.e., FEV_1) over time without evidence of sensitization is thought to be related to chronic inflammatory response in lung airways. Thus, staying

below the irritation/sensitization threshold dose should also be sufficient to avoid this adverse health effect.

In work with PMDI the pulmonary irritation-sensitization threshold has been shown to also hold for intermittent subacute and subchronic exposures in animal models (Reuzel et al., 1994; Pauluhn et al., 1999; Kilgour et al., 2002; Pauluhn, 2004). Presumably as long as the peptides and proteins in the epithelial lining fluid of the upper airways are able to sufficiently regenerate between the intermittent exposures, inhaled PMDI will be scrubbed before reaching the lower airways. This $C \times t$ relationship also appears to hold for TDI for acute to intermittent subchronic exposures as well (Schupp and Collins, 2012). The majority of the animal exposure studies with TDI found NOAELs for respiratory sensitization in the range of 5 to 30 ppb, whereas the LOAELs were about 20 to 400 ppb. Respiratory irritation NOAELs ranged mostly from 5 to 260 ppb, whereas the LOAELs ranged from 10 to 3100 ppb.

In addition to the Brown Norway rat model employed by Pauluhn (2014), some of the best evidence for a sensitization or asthmatic-like response threshold is in guinea pigs where much of the work on TDI sensitization and hyperresponsiveness has been carried out (Table 19). The data presented as total dose (ppm × min) suggests a sensitizing threshold total dose around 21.6 ppm × min for intermittent daily exposures.

Table 19 Total daily inhalation dose, in ppm × min, and response in published acute, subacute, and subchronic TDI studies using guinea pigs as the animal model.

Inhalation induction protocol (Reference)	**Dose per day (ppm × min)**	**Response**
0.020 ppm, 3 hr/d for 5 d (Aoyama et al., 1994)	3.6 ppm × min	No change
0.020 ppm, 6 hr/d, 5 d/wk for 10 wks (Karol, 1983)	7.2 ppm × min	No change
0.020 ppm, 6 hr/d, 4 d/wk for 14 wks (Wong et al., 1985)	7.2 ppm × min	No change
0.12 ppm, 3 hr/d for 5 d (Karol, 1983)	21.6 ppm × min	No change
0.023 ppm continuous for 7 d (Gagnaire et al., 1988)	233 ppm × min	↑ hyperresponsiveness
0.20 ppm, 3 hr/d for 5 d (Aoyama et al., 1994)	36 ppm × min	↑ respiration, IgG
0.36 ppm, 3 hr/d for 4 d (Karol, 1983)	64.8 ppm × min	↑ hypersensitivity
0.118 ppm continuous for 48 hrs (Gagnaire et al., 1988)	340 ppm × min	↑ hyperresponsiveness

One of the most difficult issues to contend with concerns individuals previously sensitized to TDI through occupational exposure or some other source. Once

sensitization has occurred, exposure to even exceedingly low concentrations of TDI below threshold limit values set by OSHA and other governmental agencies may precipitate symptoms (Redlich and Karol, 2002; Redlich et al., 2007). Challenge studies in sensitized workers have found exposures as low as 1 ppb of diisocyanate can cause an asthmatic response in some workers (O'Brien et al., 1979b; Lemiere et al., 2002). The lowest level of exposure in a published report resulting in an asthmatic reaction is 0.51 µg/m^3 (equivalent to 0.05 ppb) for a worker with MDI-induced asthma (Suojalehto et al., 2011). The question then becomes, "Can the RELs for TDI also protect sensitized individuals from adverse health effects resulting from TDI emissions?"

This issue can be addressed, in part, as a risk estimate by estimating the number of individuals in a population that are sensitized to TDI and other diisocyanates. If the number is exceedingly small, the risk of a sensitized person being exposed to TDI emissions under a Hot Spots scenario could be largely discounted. Very little information could be found to estimate the number of diisocyanate-sensitized individuals in a population. A review of 609 workers' compensation claims in Ontario, Canada, between 1984 and 1988 revealed that diisocyanates were the cause of 57% (135/235) of all accepted occupational asthma claims (Tarlo et al., 1995; Ribeiro et al., 2014). Irritant-induced asthma (i.e., RADS) made up approximately 5% (12/235) of these claims, and in seven of the 12 cases, TDI specifically or isocyanates was/were the irritant agent(s) (Chatkin et al., 1999). Extension of the claims review period showed that introduction of a medical surveillance program for diisocyanate workers correlated to a drop in the rate of diisocyanate irritant-induced occupational asthma claims, out of the total accepted occupational asthma claims, from a high of 64% in 1988 to 37% between 1998 and 2002. Aside from the surveillance program, other possible contributing factors to this decrease could include reduced exposure and increased awareness of diisocyanate-induced asthma by workers and physicians.

A similar review 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. Of the 30 diisocyanate claims, the specified agent was TDI (10/30), MDI (10/30), HDI (8/30), or unnamed (2/30). 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 diisocyanate sensitized due to occupational exposure is about 12 individuals per million [((135-12) + 30)/12 million].

Although similar population estimates have not been conducted in the United States, Verschoor and Verschoor (2014) reported that in the U.S. 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 U.S. population (http://quickfacts.census.gov/qfd/states/06000.html) and that no less

than 5% of those potentially exposed to TDI and possibly other diisocyanates could become sensitized to TDI at some point during their work history (Redlich et al., 2007), the frequency of sensitization due to occupational diisocyanate exposure would be approximately 43 individuals per million (1680/38.8 million). This calculation assumes an equal distribution of diisocyanate workers in California compared to the U.S. as a whole.

The limited data suggest that the number of potentially sensitized individuals in a population (i.e., 12 to 43 per million) is likely very low. This small at risk population is taken into account in deriving the RELs below. Not included in this estimate is the potential for exposure and sensitization to thermal degradation products of TDI. TDI and other related compounds generated from thermal degradation of polyurethane represent an unrecognized and often unanticipated hazard (Lockey et al., 2015).

9.1. Toluene Diisocyanate Acute Reference Exposure Level

Study	Baur et al., 1994; Vogelmeier et al., 1991
Study population	15 asthmatic and 10 healthy volunteers with no previous contact with diisocyanates
Exposure method	Exposure chamber
Continuity	
Asthmatic subjects	1 hr at 10 ppb (71 µg/m^3), 45 min break, then 1 hr at 20 ppb (142 µg/m^3)
Healthy subjects	2 hr at 20 ppb
Critical effects	≥100% increase in Raw in asthmatics
LOAEL	71 µg/m^3 (10 ppb)
NOAEL	Not determined
Time-adjusted exposure	71 µg/m^3
LOAEL uncertainty factor	10 (for severe effect)
Interspecies uncertainty factor	
Toxicokinetic (UF_{a-k})	1
Toxicodynamic (UF_{a-d})	1
Intraspecies uncertainty factor	
Toxicokinetic (UF_{h-k})	1
Toxicodynamic (UF_{h-d})	√10 (protect children with asthma)
Cumulative uncertainty factor	30
Reference Exposure Level	2 µg/m^3 (0.3 ppb)

Acute Reference Exposure Levels (RELs) are levels at which infrequent one-hour exposures are not expected to result in adverse health effects. The acute REL for TDI is intended to protect 1) individuals from acute sensory irritation and respiratory inflammation, 2) non-sensitized asthmatics from asthmatic episodes, and to some extent, 3) those individuals that are already sensitized to TDI.

The key study presented in both Baur et al. (1994) and Vogelmeier et al. (1991) was among the few controlled human exposure studies in which the total dose of TDI was sufficient to elicit sensory irritation in normal and asthmatic subjects, and an asthmatic response in some non-sensitized asthmatic subjects. A supporting study by Fruhmann et al. (1987) observed similar results in asthmatic subjects using the same exposure protocol, but it is not clear if this data is from the same group of exposed individuals described in the key studies. Concentration × duration ($C \times t$) studies in TDI-sensitized subjects observed that bronchial responsiveness was neither exclusively concentration- nor duration-dependent (Vandenplas et al., 1993). Related to $C \times t$ studies in animal models, the product of the two factors, i.e., total dose, was the main determinant for bronchial responsiveness. This could explain why shorter duration exposure studies of 15-30 min with similar TDI concentrations did not elicit effects in non-sensitized asthmatics, but did with the longer exposure durations used in the key studies.

Pulmonary function in healthy subjects was not impaired with 2 hr exposure to 20 ppb TDI, although 3 subjects reported sensory irritation (Vogelmeier et al., 1991; Baur et al., 1994; NRC, 2004). In asthmatics, 1/15 experienced a severe pulmonary response to 10 ppb during 1 hr of exposure, and 1/13 remaining asthmatics experienced a severe response to 20 ppb TDI after a 45 min break. Five of 15 asthmatics reported pulmonary symptoms of chest tightness, rhinitis, cough, dyspnea, throat irritation, and/or headache during exposure. The concentration at which these symptoms occurred was not specified in the report. Fruhmann et al. (1987) reported that the exposures to TDI at 10-20 ppb resulted in three of 15 asthmatic subjects experiencing a maximum Raw value >100% of their control value taken before exposure. An additional five asthmatic subjects had a maximum increased Raw between 50-100% of their control value,

Another factor to consider for the asthmatic response in the two subjects exposed to 10 or 20 ppb TDI in the key studies is their higher sensitivity to non-specific challenge testing with acetylcholine (Ach). Three subjects showed an increase in Raw of 100% (PD_{100}) when exposed to <0.1 mg of Ach. Two of these subjects subsequently responded with an increase in Raw of >100% when challenged with TDI. All other asthmatic subjects had a PD_{100} for Raw from exposure to 0.1-0.4 mg Ach (5 subjects) or 0.4-1 mg Ach (7 subjects). Both cross-sectional and longitudinal studies have shown a general relationship between the degree of bronchial responsiveness with certain non-specific pharmacological/physical stimuli and the severity of asthma (Josephs et al., 1990). However, how non-specific bronchial responsiveness extends to chemical irritants such as diisocyanates is less clear.

OEHHA concludes that a greater sensitivity to the acute irritant effects of TDI can occur in some asthmatic individuals because of the following lines of evidence in the key studies:

- a significant pulmonary function decrement ($\geq$100% increase in Raw) in two of 15 non-sensitized asthmatic subjects exposed to TDI

- the higher sensitivity, relative to others in the study, of these two asthmatic subjects to non-specific challenge with Ach
- an increase in Raw between 50-100% in five additional asthmatic subjects exposed to TDI
- the higher total inhalation dose (i.e., $C \times t$) used compared to most other studies exposing non-sensitized asthmatics to TDI
- the subjective symptomology of chest tightness, rhinitis, cough, dyspnea, throat irritation, and/or headache experienced by several asthmatic subjects from TDI exposure

The LOAEL from this study (with no NOAEL observed), and point of departure for REL derivation, is 10 ppb (71 µg/m^3) for a 1 hour exposure resulting in one of 15 asthmatic subjects exhibiting asthmatic symptoms (100% increase in Raw). A LOAEL-to-NOAEL uncertainty factor (UF) of 10 is applied for a severe effect (onset of asthma symptoms) occurring at the LOAEL.

No time adjustment was applied because the LOAEL-to-NOAEL uncertainty factor at least, in part, addresses this. Raw increased in exposed asthmatic subjects over the 1 hr exposure to 10 ppb TDI, with the exception of one responder due to the intensity of his response. Once exposure ended, recovery occurred in almost all individuals by 45 min.

A default toxicokinetic UF=1 was applied to the NOAEL based on the key human study that examined a sensitive subpopulation (i.e., asthmatic subjects). It is not anticipated that there would be significant interindividual variability in toxicokinetics for TDI, which acts at the portal of entry. TDI primarily affects the tracheobronchial region of the respiratory system. Pharmacokinetic computations show that children have lower tracheobronchial regional gas doses compared to adults (OEHHA, 2008) . The tracheobronchial minute volume (MV) to surface area (SA) is equal to 1 for adults, while children age 0 to 2 years have a MV/SA ratio of 0.5. For older children age 2 to 15 years, the MV/SA ratio is roughly 0.8.

The toxicogenomics data for diisocyanates show gene variants associated with increased sensitivity up to10-fold greater in workers developing diisocyanate-induced asthma. However, these findings address long-term exposures resulting in diisocyanate-induced asthma and are applied to the 8-hour and chronic REL derivations below. An intraspecies toxicodynamic default UF of $\sqrt{10}$ was used to address any potential increased sensitivity of children with asthma compared to adults with asthma. The total UF of 30, when divided into the point of departure of 10 ppb, generates an acute REL of 0.3 ppb (2 µg/m^3).

Some individuals in a population may have been previously sensitized to TDI or other diisocyanates from some other source. Once primary sensitization occurs it is probably not possible to identify a no-effect level to protect all individuals that acquired specific hypersensitivity to diisocyanates (Redlich and Karol, 2002; Redlich et al., 2007). The same conclusion was presented in an International

Consensus Report on Isocyanates (ICRI, 2002). As described above, the number of potentially sensitized individuals in the California population is likely very low, perhaps on the order of 12 to 43 per million. Two studies found that the lowest measured TDI concentration at which some sensitized individuals responded was 1 ppb (7 µg/m^3). This level is above the acute REL of 0.3 ppb. However, one report was found of an MDI-sensitized individual responding at 0.5 µg/m^3 (0.05 ppb). Keeping in mind that the RELs cannot be designed to protect all hypersensitive individuals in a population, and the likelihood that the risk of a sensitized individual being exposed to TDI emissions from a facility is very low, the acute REL is acceptable for the purposes of the Hot Spots program.

In view of the concern for sensitization due to repeated exposures to TDI (which is discussed further in the derivation of the 8-hour and chronic RELs), it is appropriate to also consider whether repeated acute exposures at the acute REL level could cause sensitization. Repeated exposure to TDI generally on the order of months to years, but sometimes weeks, is observed to result in sensitization in a small percentage of workers and subsequent induction of an asthmatic state. The acute REL is designed for infrequent 1-hour exposures. There is no evidence that infrequent exposures as low as 0.3 ppb will result in sensitization, and it is unknown if this pattern of infrequent exposure can initiate and promote sensitization. The data in animal models that shows an acute threshold dose that protects against pulmonary irritation is also sufficient to protect against sensitization would indicate that occasional exposure to the acute REL is unlikely to result in sensitization. Additionally, the acute REL is 3-fold lower than the NOAEL of 0.9 ppb upon which the 8-hour and chronic RELs rely on as the point of departure for REL derivation. Given these considerations, the acute REL is expected to be reasonably protective against sensitization under a scenario of infrequent exposures.

9.2. Toluene Diisocyanate 8-hour Reference Exposure Level

Study	Diem et al., 1982
Study population	277 adult male workers in TDI production
Exposure method	Discontinuous whole-body inhalation exposure to 0.9 or 1.9 ppb
Continuity	8 hours per day, 5 days/week
Duration	5 years
Critical effects	Accelerated decline in FEV_1
LOAEL	13.5 µg/m^3 (1.9 ppb)
NOAEL	6.4 µg/m^3 (0.9 ppb)
Time-adjusted exposure	4.571 µg/m^3 (6.4 × 5/7)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	√10
Interspecies uncertainty factor	
Toxicokinetic (UF_{a-k})	1
Toxicodynamic (UF_{a-d})	1
Intraspecies uncertainty factor	
Toxicokinetic (UF_{h-k})	10
Toxicodynamic (UF_{h-d})	10
Cumulative uncertainty factor	300
Reference Exposure Level	0.015 µg/m^3 (0.002 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 TDI is intended to protect individuals from 1) accelerated lung function decrements not related to TDI-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 TDI through occupational exposure or some other source.

The occupational study by Diem et al. (1982) used as the basis of the 8-hour REL is the same as that used for the chronic REL. The critical effect of accelerated decline in FEV_1 in the absence of TDI-induced asthma strongly indicates a chronic inflammatory response in the lung airways of the workers resulting in the loss of pulmonary function. The justification for using the key study is described in detail in the chronic REL derivation summary.

A time-adjustment of only 5 days / 7 days was applied for the 8-hour REL, since daily exposures in the critical study were 8 hours/day, 5 days/week. Support for using this time adjustment comes from $C \times t$ studies in TDI-sensitized subjects, where it was observed that bronchial responsiveness was neither exclusively concentration- nor duration-dependent (Vandenplas et al., 1993). Similar to this, acute, subacute and subchronic $C \times t$ studies in animal models, as outlined above, support equal dependence on both concentration and exposure duration for defining a threshold dose for pulmonary irritation and sensitization. Also,

chronic rodent exposure studies with MDI and PMDI strongly indicate that some level of airway recovery occurs with 6-hour per day exposures compared to exposures of 18 hours per day (Feron et al., 2001). These findings suggest a duration-dependent component for pulmonary irritation, inflammation and the induction of asthma that would support an 8-hour REL different (and higher) than the chronic REL, which is based on annualized average exposure.

A 3-fold subchronic uncertainty factor is applied to both the 8-hour and chronic RELs, since the critical study was only 5 years long. Supporting animal data shows that long-term, repeated daily TDI exposures similar to what would occur in workers may worsen airway lesions with increasing exposure duration (Loeser, 1983; Owen, 1984). Further details supporting use of individual uncertainty factors for the 8-hour REL are discussed in the chronic REL derivation below.

The critical study and other recent supporting evidence, particularly Gui et al. (2014), indicate that a low prevalence of symptoms among workers exposed to 0.5-5 ppb TDI still occurs even in state-of-the-art facilities designed to limit exposure. In the face of the animal data that suggests otherwise, there is currently no known minimum level of exposure to TDI for humans below which sensitization and asthma will not occur in susceptible individuals (Tarlo and Liss, 2002). Other issues that make it difficult to define a threshold for sensitization include dermal exposure in workers, and the large variation in the toxicogenomic response of sensitized vs. non-sensitized diisocyanate workers. Dermal exposure that may augment systemic sensitization in workers is not expected to be an issue for the Hot Spots program. However, the up to 10-fold difference in certain gene variants associated with increased sensitivity between sensitized workers and exposed workers that did not become sensitized suggest a large variation in the human population for intraspecies differences. The supporting evidence for 8-hour and chronic RELs also protecting the general public from TDI-induced sensitization is discussed below in the chronic REL derivation.

9.3. Toluene Diisocyanate Chronic Reference Exposure Level

Study	Diem et al., 1982
Study population	277 adult male workers in TDI production
Exposure method	Discontinuous whole-body inhalation exposure to 0.9 or 1.9 ppb
Continuity	8 hours per day, 5 days/week
Duration	5 years
Critical effects	Accelerated decline in FEV_1
LOAEL	13.5 µg/m^3 (1.9 ppb)
NOAEL	6.4 µg/m^3 (0.9 ppb)
Time-adjusted exposure	2.285 µg/m^3 (6.4 * 10/20 *5/7)
LOAEL uncertainty factor	1
Subchronic uncertainty factor	√10
Interspecies uncertainty factor	
Toxicokinetic (UF_{a-k})	1
Toxicodynamic (UF_{a-d})	1
Intraspecies uncertainty factor	
Toxicokinetic (UF_{h-k})	10
Toxicodynamic (UF_{h-d})	10
Cumulative uncertainty factor	300
Reference Exposure Level	0.008 µg/m^3 (0.001 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 (see Section 7 in the Technical Support Document (OEHHA, 2008)). Analogous to the 8-hour REL for TDI, the chronic REL is intended to protect individuals from, 1) accelerated lung function decrements not related to TDI-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 TDI through occupational exposure or some other source.

The chronic REL is based on a prospective occupational study by Diem et al. (1982). This study had several strengths over the other workplace investigations of TDI exposure, including minimal co-exposure to other irritating chemicals, extensive use of personal exposure monitoring devices, accounting of TDI-sensitized workers in the cohort, and in particular, detailed longitudinal analysis of workers from the start of exposure in a new TDI production facility.

In this five year longitudinal study by Diem et al. (1982), FEV_1, Forced Percentual Expiratory Volume ($FEV_{\%}$), and $FEF_{25-75\%}$ annual declines were significantly related (after controlling for smoking and atopic status) to TDI dose, where dose was measured by either: (a) two cumulative exposure categories, equivalent to a mean exposure of 0.9 or 1.9 ppb; or (b) two “time above 20 ppb” categories (division point = 0.19 months). The accelerated decline in FEV_1 was present in the absence of workers with occupational asthma. A NOAEL of 0.9 ppb

(6.4 µg/m^3), the arithmetic mean of the non-smoking low-exposure group (≤68.2 ppb-months), was calculated by Hughes (U. S. EPA, 1995). The LOAEL of 1.9 ppb is the calculated arithmetic mean of the non-smoking high-exposure group. Other longitudinal studies support the findings by Diem et al. that keeping 8-hr TWA TDI exposures below a range of about 1-2 ppb does not result in a measurable accelerated decline of pulmonary function.

OEHHA's noncancer TSD (OEHHA, 2008) recommends a 3-fold subchronic uncertainty factor for exposures from 8 to <12% of a lifetime. The 5 year duration of the Diem study represents only about 7% of a worker's lifetime of 70 years. In consideration of the generally moderate but variable amount of time required for symptom manifestation, a subchronic UF of √10 was applied (rather than a UF of 10). Supporting animal data for a subchronic UF show that chronic TDI exposure leads to a progression of upper airway lesions in female rats over the last 6 months of a 2-year exposure study (Loeser, 1983; Owen, 1984).

Since the critical study is a human study, no interspecies adjustments were required.

Due to a paucity of toxicokinetic data for TDI, such as physiologically-based pharmacokinetic modeling, OEHHA by default uses an intraspecies toxicokinetic uncertainty factor ($UF_{h\text{-}k}$) of up to 10 (OEHHA, 2008). The toxicogenomics data for TDI and other diisocyanates show gene variants associated with increased sensitivity up to10-fold greater in workers that were diagnosed with diisocyanate-induced asthma. Thus, an intraspecies toxicokinetic uncertainty factor ($UF_{h\text{-}k}$) of 10 is applied.

The intraspecies $UF_{H\text{-}d}$ (toxicodynamics) is used to account for pharmacodynamic variability among humans, including pregnant women and their fetuses and infants, children, and adults. In addition to toxicogenomics data addressing toxicokinetic variability, increased odds of developing isocyanate-induced asthma were associated with a number of genes related to toxicodynamic variability. Examples include genes involved in immune regulation, inflammatory regulation, and antioxidant defense. Although the critical effect was in an adult worker population, the potentially greater sensitivity for lung function impairment in the developing lungs of infants and children would support an intraspecies $UF_{H\text{-}d}$ of 10. The total intraspecies UF equals 100. The cumulative UF of 300 results in a chronic REL of 0.008 µg/m^3 (0.001 ppb).

Application of an overall intraspecies UF of 100 is supported by the toxicogenomic data in Table 17, in which odds ratios for immune/inflammatory system genotype variation associations for increased susceptibility for diisocyanate-induced asthma fall in the range of 1.89 to 10.36. These associations are for development of TDI-induced asthma rather than the critical effect of decreased lung function upon which the 8-hour and chronic RELs are based. The animal data supports a similar threshold total dose for both pulmonary inflammation and sensitization, which implies that a threshold for

prevention of accelerated lung function decline (likely a result of chronic airway inflammation) will also prevent pulmonary inflammation and sensitization.

Limitations exist in using occupational data for REL derivation. Seemingly at odds with the threshold dose concept for pulmonary irritation and sensitization, Weill et al. (1981) implies that a few individuals with low TDI exposure became clinically sensitized to TDI. Gui et al. (2014) also found that even though exposure is often below the limit of detection (0.1 ppb) and almost never goes above 5 ppb, some workers still exhibited occupational asthma-like effects and other symptoms related to TDI exposure, although overall pulmonary function of the cohort is unaffected. The occupational environment may have exposure scenarios that promote sensitization, but that are unlikely to occur with residential or off-site workplace exposures to airborne emissions from a Hot Spots facility. These occupational scenarios include the potential for dermal exposure and inhalation exposure to occasional brief high concentrations (e.g., accidental spills) that surveillance monitoring may miss. Dermal exposure to TDI has been shown to lead to systemic sensitization in animals, rendering them "hypersensitive" to induction of asthma-like symptoms with subsequent TDI inhalation exposure. The 8-hour and chronic RELs may be over-protective if these occupational conditions are major contributors to TDI-induced asthma. However, it is unknown how much of a factor short-term, high inhalation exposures and dermal contact to diisocyanates are for initiation of sensitization and induction of asthma in humans.

The 100-fold intraspecies UF, based mainly on gene variants associated with increased sensitivity in workers that were diagnosed with diisocyanate-induced asthma, suggest a wide variation in response among the human population. The large intraspecies UF accounts for the uncertainty in establishing a minimum level of TDI exposure that will not lead to sensitization in susceptible individuals. What is certain is that the proportion of exposed workers who become sensitized is reduced when TDI exposure levels are reduced in the workplace (Tarlo et al., 1997; Meredith et al., 2000; Ott et al., 2000; Tarlo and Liss, 2002; Gui et al., 2014). Given these findings and consideration that the RELs are not designed to protect every hypersensitive or hyperresponsive individual in a population, public health is sufficiently protected with the OEHHA 8-hour and chronic RELs.

Some individuals in a population may have been previously sensitized to TDI or other diisocyanates from some other source(s). Once primary sensitization occurs it is probably not possible to identify a no-effect level to protect all individuals that acquired specific hypersensitivity to diisocyanates (Redlich and Karol, 2002; Redlich et al., 2007). The same conclusion was presented in an International Consensus Report on Isocyanates (ICRI, 2002). As described above, the number of potentially sensitized individuals in the California population is likely very low (e.g., 12 to 43 per million). Two studies found that the lowest measured TDI concentration at which some sensitized individuals responded was 1 ppb (7 $\mu g/m^3$) (O'Brien et al., 1979b; Lemiere et al., 2002). One report exists of an MDI-sensitized worker responding at 0.05 ppb

(Suojalehto et al., 2011). The 8-hour and chronic RELs of 0.002 and 0.001 ppb, respectively, are lower than the lowest level resulting in sensitized individuals responding. Keeping in mind that the RELs are not designed to protect every sensitized individual in a population, and the likelihood that the risk of a sensitized individual being exposed to TDI emissions from a facility is very low, the 8-hour and chronic RELs are appropriate for the purposes of the Hot Spots program.

9.3.1. Comparison RELs

The U.S. EPA (1995) estimated a Reference Concentration (RfC) of 0.07 μg/m^3 for TDI, analogous to the OEHHA chronic REL, also based on the Diem et al. for chronic lung function decline. The RfC does not account for exposure that could lead to sensitization, nor does it account for those individuals already sensitized. The 10-fold difference in the U.S. EPA derivation and the chronic REL results from OEHHA's use of intraspecies toxicodynamic and toxicokinetic UFs of 10, rather than √10, to address the respiratory susceptibility of children, and the differences in risk of diisocyanate-induced asthma in workers based on the toxicogenomic data.

Pauluhn (2014) derived a human worker exposure level for respiratory tract irritation and prevention of sensitization based on an "asthmatic" rat model. A detailed presentation of this model is described in Section 5.3. OEHHA used the rat respiratory tract irritation/sensitization threshold to derive RELs and compare it with the RELs derived above based on human data. The threshold used as the point of departure, defined as 1000 mg/m^3 × min, is the total dose at which an asthmatic-like response of allergic pulmonary inflammation is not initiated in rats previously sensitized to TDI. The critical effect in the rats is increased neutrophilic granulocytes (PNMs) in BALF, an effect also observed in sensitized workers exposed to TDI.

To derive an acute REL, 1000 mg/m^3 × min is divided by 60 min to determine a concentration of 16.7 mg/m^3 as the point of departure. OEHHA could justifiably apply the dosimetric adjustments developed by Pauluhn (2014) of √10 for obligate vs. oronasal breathing, and √10 for the assumption that humans may not depress their respiration rate and minute volume as rats do with exposure to irritant doses of TDI. To these interspecies toxicokinetic adjustments, OEHHA would also include a default interspecies toxicodynamic uncertainty factor of √10. For intraspecies uncertainty, OEHHA would use a 30-fold factor (10 for toxicokinetic and √10 for toxicodynamic) for variability in the human population, not just a worker population as Pauluhn used in his derivation. The total uncertainty adjustment factor would then = 1000 (√10 × √10 × √10 × √10 × 10). The OEHHA-derived comparison acute REL is 0.0167 mg/m^3 (16.7 μg/m^3 or 2.4 ppb). Use of human data to derive the acute REL results in an 8-fold lower value of 2 μg/m^3 (0.3 ppb), a more health-protective level for the REL.

For an 8-hour REL based on the same endpoint and threshold of 1000 mg/m^3 × min, OEHHA would use an 8-hour time adjustment and the same interspecies factors as used in the acute REL derivation. OEHHA uses a 100-fold intraspecies uncertainty factor (10 each for toxicokinetic and toxicodynamic UFs) based mainly on gene variants associated with increased sensitivity in workers that were diagnosed with diisocyanate-induced asthma. The comparison 8-hr REL would result in a value of 0.0007 mg/m^3 (1000 mg/m^3 × min / (480 min × 3000)), equivalent to 0.7 µg/m^3 (0.1 ppb). Use of human data to derive the 8-hour REL results in a roughly 50-fold lower value of 0.015 µg/m^3 (0.002 ppb), a more health-protective level for the REL. To derive a chronic REL, a time adjustment of 1440 min would be used. Applying the same dosimetric adjustments and uncertainty factors as used in the 8-hour REL derivation, a chronic REL of 0.2 µg/m^3 (0.03 ppb) is calculated.

Pauluhn (2014) used the NOAEL of 1000 mg/m^3 × min in his rat model to derive a human time-adjusted 8-hour worker exposure level. The author divided 1000 mg/m^3 × min by 480 min, and applied the dosimetric adjustments of √10 for obligate vs. oronasal breathing, and √10 for the assumption that humans may not depress their respiration rate and minute volume as rats do with exposure to irritant doses of TDI. Pauluhn also applied an intraspecies uncertainty factor = 10. Thus, 8-hour adjusted worker NOAEL of 0.02 mg/m^3-day (20 µg/m^3, 3 ppb) is calculated (1000 mg/m^3 × min / (480 min × √10 × √10 × 10)).

9.4. TDI 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 when developing Reference Exposure Levels for TACs. TDI was identified by the Air Resources Board as a toxic air contaminant in accordance with Section 39657(b) of the California Health and Safety Code (Title 17, California Code of Regulations, section 93001) (CCR, 2007). TDI has been shown to cause asthmatic reactions in non-sensitized asthmatic adults in controlled exposure studies (Vogelmeier et al., 1991; Baur et al., 1994), and possibly in non-sensitized children with asthma as well as causing asthma-like effects in children without asthma who were exposed acutely to a MDI/xylene mixture in an accidental exposure (Jan et al., 2008). 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). In view of the potential of TDI to induce or exacerbate asthma and the differential impacts of asthma on children including higher prevalence rates, and in view of the rapid development of the lung during infancy, OEHHA recommends that TDI be identified as a TAC that may disproportionally impact children pursuant to Health and Safety Code Section 39669.5(c).

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