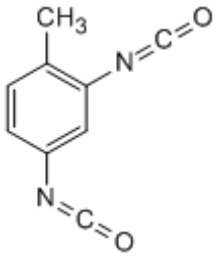
Toluene diisocyanate

***(*1,3-Diisocyanatomethylbenzene, 2,4- and 2,6-Toluene diisocyanate, Methyl-m-phenylene diisocyanate, Methylphenylene isocyanate, Tolylene diisocyanate, TDI*)**

CAS: 26471-62-5



1 Summary

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

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

1.1 Toluene diisocyanate Acute REL

Reference exposure level	0.5 $\mu g/m^3$ (0.07 ppb)
Critical effect(s)	Respiratory rate depression
Hazard index target(s)	Upper respiratory tract

1.2 Toluene diisocyanate 8-hour REL

Reference exposure level	0.02 μg/m^3 (0.003 ppb)
Critical effect(s)	Decreased lung function
Hazard index target(s)	Lower respiratory tract

1.3 Toluene diisocyanate Chronic REL

Reference exposure level	0.02 μg/m^3 (0.003 ppb)
Critical effect(s)	Decreased lung function
Hazard index target(s)	Lower respiratory tract

2 Physical & Chemical Properties (HSDB 2004)

	CAS	Vapor pressure
Toluene diisocyanate	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
Melting point	11-14°C
Vapor pressure	2.3 x 10^{-2} mm Hg @ 25°C
Odor threshold	Pungent odor
Solubility	Alcohol, acetone, carbon tetrachloride, benzene, kerosene.
Conversion factor	7.1 mg/m^3 = 1 ppm @ 25° C

3 Major Uses and Sources

Toluene diisocyanate (TDI) is used in adhesives, coatings, elastomers, and polyurethane foams. During its production and use, it may be released to the environment by volatilization and through various waste streams. Reported release of TDI to the air in California in 2008 was at the rate of 0.28 tons/year (CARB 2008). Commercial TDI is an isomeric mixture typically comprising 80% 2,4-toluene diisocyanate and 20% 2,6-toluene diisocyanate. Given the vapor pressures of the constituent isomers, in air, toluene diisocyanate will exist solely as a vapor. Vapor-phase TDI may be degraded in the atmosphere by reaction with photochemically-produced hydroxyl radicals with an estimated half-life of 2.7 days for the mixed isomers, 1.7 days for 2,4-TDI, and 2.5 days

for the 2,6-isomer. Because TDI reacts with water, atmospheric degradation may also occur through contact with clouds, fog or rain. At low levels of TDI, rapid degradation in water is expected to preclude leaching or adsorption to solids in moist soils, as well as bioconcentration in aquatic organisms.

Occupational exposure to toluene diisocyanate may occur through inhalation and dermal contact during its production or use. The general population may be exposed to toluene diisocyanate via use of consumer products containing this compound. These may include products in which the monomeric or prepolymeric form of TDI is present by design, such as in paints. However, in an attempt to minimize potentially harmful exposures, TDI has been replaced in many applications, especially consumer products, by other less volatile and reactive compounds such as methylene diphenyl diisocyanate (MDI) and hexamethylene diisocyanate (HDI), so direct handling of TDI containing materials by consumers is rare. Exposure of the general population to low levels may occur from finished polyurethane products in which traces of unreacted TDI remains following manufacture. These include foam in bedding and furniture, in medical devices and bandages. During the manufacture of polyurethane foam, isocyanate is added in excess of that needed for the stoichiometric reaction that creates the polymer to speed the curing process. As a result, unreacted isocyanate monomers may remain in the foam and be slowly released. Indeed, Krone et al. (2003) applied semiquantitative tests for isocyanate to polyurethane products, including mattresses, mattress pads, sofa padding, carpet pads and pillows, and detected free isocyanate in products up to 30 years after manufacture.

These characteristics refer to TDI in its monomeric form. However, TDI is also manufactured as a prepolymer (Figure 1) which is much less volatile but 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.

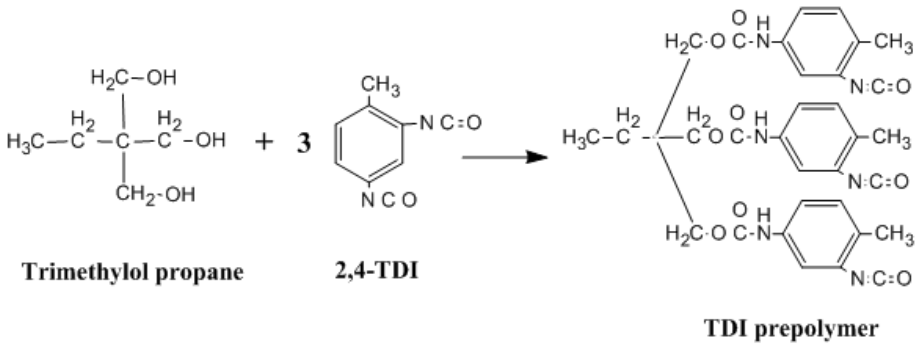


Figure 1. Formation of TDI Prepolymer

4 Metabolism

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 including hemoglobin, glutathione, laminin, serum albumin, and tubulin (Brown and Burkert 2002). In the gut, hydrolysis of TDI generates toluene-2,4-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. In experimental administration by the oral route, 12-20% of the dose was absorbed, while by the inhalation route, essentially all the TDI was retained (Timchalk et al., 1994). Inhalation exposure leads preferentially to the formation of TDI conjugates and much less TDA. These route-dependent differences in fate likely explain the carcinogenic potential of TDI by the oral (with conversion to TDA) but not the inhalation route (Collins 2002). 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.

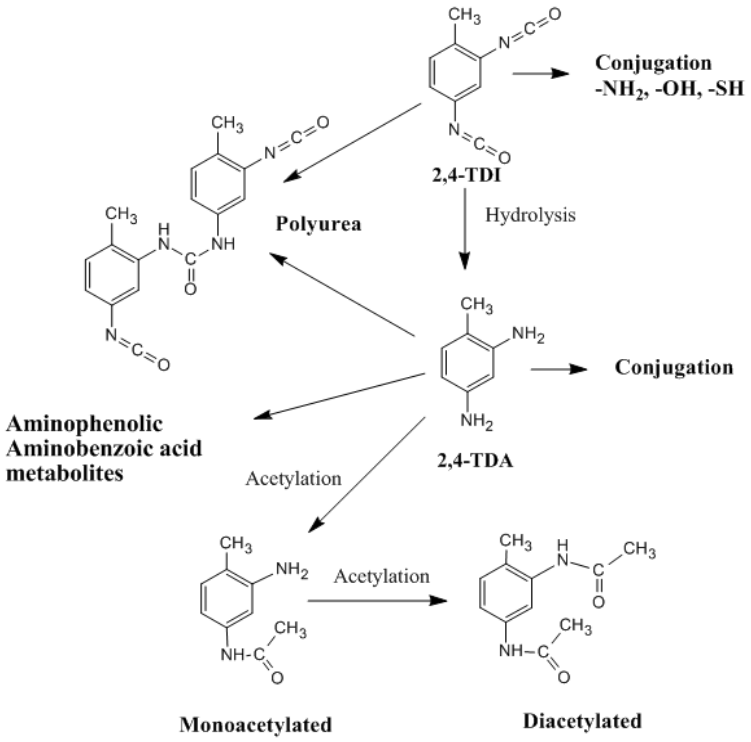


Figure 2. Metabolic Scheme for TDI in Rat (Timchalk et al., 1994)

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 is often measured as respiratory rate depression. However, TDI may also act as a pulmonary irritant resulting in increased respiratory rate (Castranova et al., 2002). Dermal exposure and/or inhalation exposures to high, or long-term lower levels of TDI may result in allergic sensitization. The sensitizing potential of TDI and other reactive electrophiles appears to be related to its ability to bind to a specific cation channel, TRPA, in sensory neurons (Macpherson et al., 2007; Taylor-Clark et al., 2009). TRPA belongs to the family of transient receptor

potential (TRP) channels that transduce sensory neurons' response to thermal, mechanical, and specific chemical stimuli. In addition to activating the neuron, TDI binding is thought to covalently modify TRPA and increase its responsiveness to subsequent stimuli. Activation of this channel in sensory neurons leads to a cascade of events including release of neuropeptides and potentiation of synapses in the brainstem. The neuropeptides released in the respiratory tract cause mast cell degranulation, contraction of airway smooth muscle, enhanced mucus secretion, leukocyte infiltration, pulmonary extravasation, and pulmonary edema (Fig. 3).

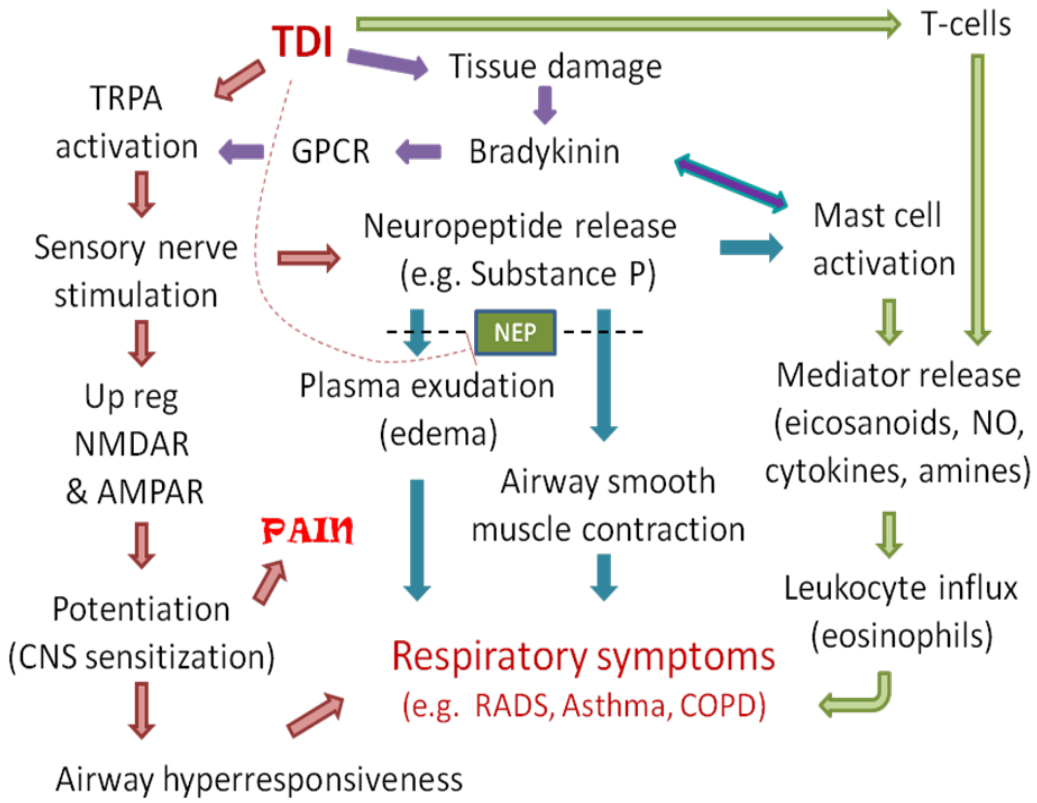


Figure 3 TDI-Stimulated Pathways Leading to Respiratory Symptoms
Diisocyanates activate TRPA channels in nociceptive neurons in the airways leading to respiratory symptoms via long-term potentiation of neural pathways, release of inflammatory mediators, and stimulation of the immune system. NEP: neutral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

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., 2000). 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). While less likely than with chronic

exposures, high acute exposure may result in allergic sensitization. In these cases, a subsequent acute inhalation exposure may provoke a potentially life-threatening pulmonary hypersensitivity response.

5.2 Acute Toxicity to Infants and Children

No studies were located that examined the effects of acute exposure to TDI in children. However, these effects may be expected to be generally similar to those reported following acute accidental exposure of school children to methylene diphenyl diisocyanate (MDI) and toluene (Jan et al., 2008). The most common immediate symptoms of exposure were headache, persistent cough, dyspnea and nausea. Reactive airway dysfunction requiring treatment with bronchodilators was reported, including among children with no previous history of asthma-like symptoms. However, respiratory symptoms were exacerbated in children with a history of asthma. It is not known whether any of the children were immunologically sensitized as a result of the exposure.

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 was 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 lumens 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 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 1, 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. The RD50 at 60 min is the point of departure for the acute REL.

Table 1 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. 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.

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) demonstrated that single dermal exposures to TDI were sufficient to elicit signs of significant pulmonary sensitivity, as measured by increases in respiratory rates greater than three 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 with TDI-protein conjugates, in 5 of 12 with toluene monoisocyanate-protein conjugates, and in none of the animals challenged with the unconjugated protein carriers alone.

6 Chronic Toxicity of Toluene diisocyanate

Chronic exposure to relatively low levels of TDI may result in allergic sensitization. Thus, perhaps the most significant long term effect associated with TDI exposure is a constellation of symptoms collectively described as diisocyanate-induced asthma (DIA), characterized by bronchial inflammation with lymphocytic infiltration and eosinophilia, airway hyperresponsiveness, and airway remodeling. Indeed, isocyanate exposure is one of the leading causes of occupational asthma (Chan-Yeung 1990).

6.1 Chronic Toxicity to Adult Humans

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. Changes in pulmonary function, measured as changes in forced expiratory volume in 1 sec (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 exposures were measured with continuous tape monitors, but not until two years into the study. 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. 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).

Among workers who never smoked, FEV_1 was significantly reduced by 38 ml/year in the high-exposure group (n = 21) compared to the low-exposure group (n = 35)(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). Similarly, in the low-exposure group, the mean annual decline in forced expiratory flow (25-75%) [$FEF_{(25-75\%)}$] was 81 ml/s-year 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/s-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. Based on the dichotomization of groups into high (1.9 ppb) and low (0.9 ppb) groups, a LOAEL of 1.9 ppb and a NOAEL of 0.9 ppb may be assigned for these pulmonary effects.

In workers occupationally exposed to TDI, elevated titers of tolyl-reactive IgE antibodies are associated with pulmonary hypersensitivity reactions. In a 13-month longitudinal study, Karol et al. (1979) measured tolyl IgE titers with a solid phase radioimmune assay (RAST), and correlated this with clinical diagnoses. Among non-sensitized, asymptomatic workers, the mean IgE titers were 219 cpm, while levels above 456 cpm were considered significantly elevated ($p < 0.01$). In this context, three workers were diagnosed as hypersensitive based on observations of clinical hypersensitivity episodes. Case 1 experienced wheezing, coughing and chest pains within three minutes following inhalation of TDI vapor. In a bronchial provocation test with 0.006 ppm TDI, his FEV1 dropped 73%. During the six months prior to the provocation test, his tolyl IgE titer rose from 1660 to 2879 cpm and he experienced several asthma attacks associated with TDI exposures. Six months following removal from exposure, this worker experienced no further asthma episodes but his IgE titers remained elevated at 1968 cpm. The second case experienced cutaneous hypersensitivity with dermal exposure to TDI that manifested as hives or maculopapular lesions over her hands and forearms. Unlike the other cases, there were no apparent pulmonary effects. Her initial IgE titer of 1050 cpm declined following removal from isocyanate exposure to 270 cpm, comparable to non-sensitive workers. The third case experienced both dermal (hives) and pulmonary (asthma) effects. At the start of the study, this worker's IgE tier was 983 cpm that rose to 1252 cpm in association with an asthma attack following exposure to a TDI spill. Although his titer gradually declined to 797 cpm, subsequent contact with a worker wearing TDI-contaminated clothing elicited an acute asthma episode that required hospitalization. This study suggests that once an individual is sensitized to TDI, the susceptibility to potentially serious hypersensitive responses may only gradually abate in the absence of repeated exposure.

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). Followup 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 (BAL), along with epithelial damage and thickening of the basement membrane (Paggiaro et al., 1990).

6.2 Chronic Toxicity to Infants and Children

No studies of inhalation exposures to TDI among children were located. However, early life exposure to TDI may occur via inhalation and dermal contact with polyurethane products. As mentioned above in Section 4, free isocyanate has been detected in and on polyurethane foam bedding (Krone et al., 2003). When this bedding is used by infants, there is a potential for exposure of the developing immune system to TDI. Indeed, among children with 12 or more wheezing attacks in the previous 12 months, independent associations have been reported 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) (Strachan and Carey 1995). It has also been observed that there is a higher incidence of

asthma among first children compared with their siblings (Karmaus and Botezan 2002). This is consistent with the use of new polyurethane products (bedding, car seats, etc.) by the first child while siblings often use hand-me-downs whose isocyanate content has diminished over time. These early life diisocyanate exposures may be significant since 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). Indeed, in animal models of TDI-induced asthma, T_H2 cytokines were found to be decisive in the initial phase of occupational asthma, in the priming of T_H2 cells, and in the permeation of eosinophils into the airway lumen (Matheson et al., 2005). In other mouse models of asthma, a T_H2-dominated sensitization was best achieved via dermal exposure followed by tracheal exposure, while exposure exclusively by inhalation prompted more of a T_H1 type response (Ban et al., 2006). Thus if a T_H2 response predominates, whether by previous activation or due to an early developmental stage, subsequent respiratory exposure may result in a substantial pulmonary inflammatory response. Other examples of chemical stressors that can shift the immune response to a T_H2 pattern are diesel exhaust and ETS, both of which are strongly associated with asthma.

6.3 Chronic Toxicity to Experimental Animals

The effects of TDI exposure are concentration- and duration-dependent. 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. In this model, 6 weeks is the period during which sensitization to TDI develops. Three control groups included air-exposed/air-challenged, TDI-exposed/air-challenged, and air-exposed/TDI-challenged mice. A portion of each group was sacrificed 24 hr after the challenge for the collection of blood and bronchioalveolar lavage fluid (BALF). Another portion was sacrificed 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, these 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.

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 may reflect pulmonary changes associated with the immune response (Karol et al., 1980). 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-GSA 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. To examine the effects of long-term, low-level exposure, animals were exposed to 0.02 ppm TDI 6 hr/day for 70 days giving a total exposure of 8.67 ppm x hr. This was comparable to the total exposure of 9.2 ppm x hr received by animals breathing 0.61 ppm for 3 hr/day for 5 days. Whereas animals receiving the higher level (0.61 ppm), short term exposure displayed bronchial hyper-responsiveness and antibody production, none of the animals with low level (0.02 ppm) TDI exposure developed antibodies or pulmonary responsiveness upon challenge. 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. However, as observed in the occupational study by Diem et al.(1982), pulmonary effects (decreased FEV_1) were associated with a low TDI concentration of 1.9 ppb.

7 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 lives 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). Forty-two-day-old Sprague-Dawley rat pups (28/sex/group) were exposed 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.

8 Derivation of Reference Exposure Levels

8.1 Toluene Diisocyanate Acute Reference Exposure Level

Study	Sangha & Alarie, 1979
Study population	120 male Swiss Webster mice
Exposure method	
Continuity	Continuous 0.08 to < 2.0 ppm
Duration	1 hr
Critical effects	50% depression of respiratory rate
RD50 1 hr exposure	0.386 ppm
Time-adjusted exposure	0.386 ppm
LOAEL uncertainty factor	n/a
Subchronic uncertainty factor	n/a
Interspecies uncertainty factor	
Toxicokinetic (UF_{a-k})	n/a
Toxicodynamic (UF_{a-d})	n/a
Intraspecies uncertainty factor	
Toxicokinetic (UF_{h-k})	n/a
Toxicodynamic (UF_{h-d})	n/a
Cumulative uncertainty factor	n/a
Reference Exposure Level	0.07 ppb (0.50 µg/m^3) (RD50 = 0.00026*0.386$^{1.4}$)

Acute Reference Exposure Levels (RELs) are levels at which infrequent one-hour exposures are not expected to result in adverse health effects. Sensory irritation and altered lung function are the main responses seen with acute exposure to TDI. Depression of respiratory rate is a function of sensory irritation. The acute REL is based on the depression of respiratory rates by 50% (RD50) in mice upon exposure to TDI for one hour (Sangha and Alarie 1979). The method for calculating an acute REL using relationships proposed by Kuwabara et al. (2007) is described below.

In a study examining the applicability of RD50 values in setting exposure levels, Kuwabara et al. (2007) found acute RELs to be related to RD50s ($R^2 = 0.71$) determined in mice via the expression REL = 0.00026 x RD50$^{1.4}$. Applying this relationship to the observed 1 hr RD50 of 0.386 ppm gives an acute REL of 0.07 ppb. No adjustments were applied since this relationship is between RD50s and previously determined RELs.

In view of the concern for sensitization by exposures to TDI (which is discussed further in the derivation of the 8-hour and chronic RELs), it is appropriate to consider whether repeated acute exposures at the acute REL level could cause sensitization. Although

acute RELs are defined for single exposures, it is possible that an acute increase in the exposure concentration might be repeated on an occasional basis, and this type of exposure pattern may promote sensitization. However, in the study upon which the cREL is based, 0.9 ppb was the NOAEL for five years of occupational exposure, so there is a greater than 10-fold difference between this level for a regularly repeated exposure and the acute REL of 0.07 ppb). Therefore the acute REL should be adequately protective for most individuals, although the possibility of sensitization of certain hypersensitive individuals cannot be excluded.

8.2 Toluene diisocyanate 8-hour Reference Exposure Level

Study	Diem et al., 1982
Study population	277 adult male workers in TDI production
Exposure method	Discontinuous occupational whole-body inhalation exposure to 0.9 or 1.9 ppb
Continuity	8 hours per day, 5 days/week
Duration	5 years
Critical effects	Decreased lung function (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	100
Reference Exposure Level	0.02 µg/m^3 (0.003 ppb)

The 8-hour Reference Exposure Level is a concentration at or below which adverse noncancer health effects would not be anticipated for repeated 8-hour exposures.

The proposed 8-hour REL is based on the prospective occupational study by Diem *et al.* (1982) of workers in a new TDI production plant. Nine measurements of FEV_1 over a 5 year period documented a decrease in lung function with TDI exposure. A NOAEL of 0.9 ppb (6.4 µg/m^3) for this effect was calculated by Hughes (U.S. EPA, 1995). The time adjustment used here is the same as for the chronic REL below. For many substances, higher exposure levels are tolerable if the exposures are intermittent versus chronic, thus 8-hr RELs are typically higher than chronic RELs. For TDI, the lower exposure level of the chronic REL was also chosen for the 8-hour REL as a more health protective approach because the pulmonary effects of TDI are the result of its sensitization of the neuroimmune system, an effect that may occur with only intermittent low-level

exposures. Sensitization to TDI that occurs following an acute, high-level exposure primarily represents a neurological sensitization with only non-specific involvement of the immune system. The development of TDI-specific IgE (and possibly IgG) antibodies requires longer term exposures and may occur at low exposure levels (Scheerens et al., 1999; Matheson et al., 2005). The manner in which airway hyperresponsiveness and remodeling occur is thus affected by the intensity and duration of exposure. For this reason, while some individuals may become sensitized to TDI in a relatively short period of time, for others, sensitization may only develop following years of low level exposure (Peters and Wegman 1975). In the former case, neural sensitization may ultimately trigger an immune system involvement, while in the latter case, development of a specific immune response can potentiate neural responsiveness. Although the 5 year duration of the study represents less than 8% of a worker's lifetime, in consideration of the generally moderate but variable amount of time required for symptom manifestation, a subchronic UF of √10 was applied. Since the critical study is a human study, no interspecies adjustments were required. For intraindividual variability an intraspecies toxicokinetic UF of √10 was applied. This was chosen since decreased lung function is not a systemic effect nor is it metabolically mediated. However, due to TDI's potential for hypersensitization and the more severe effects of lung function impairment in children, an intraspecies toxicodynamic UF of 10 was applied to give a cumulative UF of 100 and an 8-hr REL of 0.02 μg/m^3 (0.003 ppb).

8.3 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	Decreased lung function
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	100
Reference Exposure Level	0.02 μg/m^3 (0.003 ppb)

The proposed chronic REL is also based on the prospective occupational study by Diem *et al.* (1982) of workers in a new TDI production plant. Nine measurements of FEV_1

over a 5 year period documented a decrease in lung function with TDI exposure. A NOAEL of 0.9 ppb (6.4 $\mu g/m^3$) for this effect was calculated by Hughes and adjusted to a continual exposure of 2.285 $\mu g/m^3$. This was a subchronic study to which a subchronic UF of $\sqrt{10}$ was applied, as described above for the 8-hour REL. No interspecies adjustments were necessary. For intraindividual variability an intraspecies toxicokinetic UF of $\sqrt{10}$ was used since decreased lung function is not a systemic effect nor is it metabolically mediated. However, due to the potentially more severe effects of lung function impairment in children and TDI's ability to cause hypersensitization, an intraspecies toxicodynamic UF of 10 was applied to give a cumulative UF of 100 and a chronic REL of 0.02 $\mu g/m^3$ (0.003 ppb).

For comparison, the U.S. EPA (1995) estimated an RfC of 0.07 $\mu g/m^3$ also based on the Diem study. The three-fold difference in the U.S. EPA derivation and the proposed REL results from OEHHA's use of a toxicodynamic UF of 10 to address the respiratory susceptibility of children, as opposed to the default $\sqrt{10}$ for this type of interindividual variability implied in U.S. EPA's derivation.

The study upon which the REL is based did not specifically examine hypersensitivity to responses to TDI. However, because TDI exposure may result in airway hyper-responsiveness, it is worth noting that in workers with isocyanate induced asthma who were challenged at a level of 1 ppb for a total dose of 47 ppb*min isocyanates, asthmatic responses were induced in 3 of 8 subjects (Lemiere et al., 2002). This value is 10% of the sensitizing concentration. Comparing this with an acute REL of 0.07 ppb, or the 8-hr REL of 0.003 ppb, suggests that these RELs will protect against initial sensitization and a subsequent hypersensitive response.

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