Annex I to the CLH report

- non confidential -

Proposal for Harmonised Classification and Labelling

Based on Regulation (EC) No 1272/2008 (CLP Regulation), Annex VI, Part 2

International Chemical Identification:

2,4,6-triisopropyl-m-phenylene diisocyanate;

TRIDI

EC Number: **218-485-4**

CAS Number: **2162-73-4**

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Contact details for dossier submitter:

BAuA
Federal Institute for Occupational Safety and Health
Federal Office for Chemicals
Friedrich-Henkel-Weg 1-25
44149 Dortmund, Germany

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Note to the reader: For an explanation of the abbreviations used in this Annex, please refer to the list of abbreviations provided in the main dossier.

1 HEALTH HAZARDS

1.1 Respiratory sensitisation

1.1.1 Human data for the category source substances HDI, MDI, TDI

1.1.1.1 Case reports

Table 1: Cases related to HDI, MDI, and/or TDI as documented in the published literature (non-comprehensive)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Case report of three painters with respiratory tract symptoms	#1: Spray-painting with polyisocyanate lacquer #2: Painting with polyisocyanate plastic lacquer #3: Spray-painting, brush-painting with plastic lacquer	TDI	#1: Asthmatic bronchitis #2: Asthmatic symptoms/attacks #3: Not specified (severe cough, pressure on the chest)	(Swensson et al., 1955)
Case report of six subjects with respiratory symptoms suggestive of diisocyanate sensitisation	Developmental and experimental work on urethane foams and surface coatings; #1: Engineer, known to be sensitised to TDI. Re-exposure occurred unintentionally due to an accident. #2/3/4: Laboratory assistants using TDI to produce plastic foams. #5: Fitter dismantling equipment which was used in the making of foam. #6: Not accepted as a case of sensitisation as symptoms were attributed to anxiety.	TDI	TDI respiratory sensitisation as demonstrated by respiratory symptoms	(Williamson, 1965)
Examination by bronchial provocation test for sensitivity to TDI of 24 workers with respiratory disease handling diisocyanates	Not specified	HDI. MDI, TDI	Asthma	(O'Brien et al., 1979)
Study to determine the mechanisms of bronchial hyperreactivity ("sensitivity") to TDI in 28 workers with a history of sensitivity to TDI	TDI production	TDI	Asthmatic reactions; five workers were identified as non-reactors	(Butcher et al., 1979)
Case report of two workers with respiratory symptoms	Not specified #1: Production supervisor #2: Welder, exposed continuously to polyurethane foam fumes	MDI	#1: Occupational asthma #2.: Hypersensitivity pneumonitis	(Zeiss et al., 1980)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Radioallergosorbent testing of 26 TDI-reactive individuals shown to react to provocative inhalation challenge with TDI	Not specified	TDI	Asthma	(Butcher et al., 1980)
Case report of four subjects diagnosed with MDI-related asthma	Welding of polyurethane belts	MDI	Asthma	(Lob and Boillat, 1981)
Case report of subject with repeated prolonged exposure to MDI	Manufacturing engineer	MDI	Hypersensitivity pneumonitis and pleuritis progressing to fibrosing alveolitis	(Friedman, 1982)
Inhalation challenge tests in exposed workers with respiratory symptoms related to TDI or MDI	MDI: Not specified; TDI: Printers and laminators of flexible packaging	TDI, MDI	Occupational asthma in 24/40 workers with MDI- and 30/51 workers with TDI-related respiratory symptoms	(Burge, 1982)
Case report of subject with history of shortness of breath, wheezing, malaise and chills	Foreman in a garage where painting was done using a polyisocyanate activator	HDI	Combined alveolitis and asthma	(Malo et al., 1983)
Retrospective analysis of 109 MDI production workers	MDI production	MDI	8/109 workers were diagnosed with chronic obstructive bronchial disease and 3/109 with contact dermatitis.	(Diller and Herbert, 1983)
Case report of one subject	Manufacture of shoe soles	MDI	Occupational asthma	(Innocenti and Paggiaro, 1983)
Case report of one patient with symptoms of hypersensitivity pneumonitis	Packing and shipping of automobile equipment, occasionally engaged in spraying a mixture of MDI and polyol to produce polyurethane foam	MDI	Hypersensitivity pneumonitis	(Baur et al., 1984)
Case report of one patient showing symptoms of severe asthma	Grain elevator operator/repairman cutting polyurethane plate made of MDI	MDI	Occupational asthma	(Chang and Karol, 1984)
Case report of two patients with developed asthma and/or alveolitis	Painting, insulating	HDI, MDI	Asthma, alveolitis	(Laitinen et al., 1984)
Mechanistic challenge study in four subjects exhibiting a late asthmatic response after TDI exposure	Not specified	TDI	Asthma	(Mapp et al., 1985)
Case-control study in 78 workers with respiratory symptoms,.372 railway yard repair workers, representing 95% of the work force, served as negative controls.	Iron and steel foundry; workers handling PepSet, a chemical binding system containing MDI	MDI	Asthma (12/78)	(Johnson et al., 1985)
Case report of two workers who developed asthmatic symptoms	Gym-shoe factory, injecting MDI into shoe soles	MDI	#1: Asthma, hypersensitivity pneumonitis #2: Asthma	(Mapp et al., 1985)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Case report of one patient with a history of respiratory illness	Chemical industry technical representative, exposed while unloading a railroad tank car containing MDI and having further work-related intermittent exposure	MDI	Occupational asthma	(Banks et al., 1986)
Case report of one patient with asthma persisting for twelve years after single massive exposure to TDI	Not specified	TDI	Asthma	(Moller et al., 1986)
Case report of four workers with respiratory symptoms	Iron foundry; core making, sand mixing, and fettling associated with the Cold-Box process	MDI	Asthma bronchiale due to contact with isocyanates	(Erban, 1987; Erban, 1988).
Study on the inhibitive effect of prednisone on late asthmatic reactions and airway inflammation induced by TDI in eight sensitised subjects with previously documented late asthmatic reactions	Not specified	TDI	Asthmatic reactions	(Boschetto et al., 1987)
Case report of one patient having TDI-induced asthma	Accidental peak exposure during maintenance work in a chemical plant (this peak exposure lead to onset of symptoms of asthma)	TDI	Isocyanate induced Asthma. Positive in 1974 (after accident), no hyperresponsiveness to challenge testing in 1985 (after 11 years without exposure to TDI), but positive in 1987 (after return to work with TDI).	(Banks and Rando, 1988)
Case report of one patient diagnosed with asthma induced by TDI	Self-employed car painter	TDI	Death after an asthma attack The subject was recommended to cease working with isocyanates after diagnosis of asthma induced by TDI in 1980. Nevertheless he continued under usage of anti-asthmatic drugs. He died 1986 within 1 hour of the second exposure to a new kind of polyurethane paint in the workplace.	(Fabbri et al., 1988)
Challenge study examining cross-reaction between TDI and MDI in 25 subjects having developed asthma to TDI	Furniture industry, handling polyurethane varnishes catalysed with TDI	TDI	Occupational asthma	(Innocenti et al., 1988)
Case report of eight patients with an unequivocal history of professional asthma	#1: Employee in polyurethane foam car seat manufacture #2, 4, 5, 6, 7, 8: Workers in shoemaking factory #3: Shoemaker	HDI, MDI., TDI	Occupational asthma	(Cvitanovic et al., 1989)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Assessment of specific IgE and IgG antibodies in 62 workers with possible occupational asthma caused by isocyanates	Workers in foam industry (TDI), spray painters (HDI/MDI), various (MDI)	HDI, MDI, TDI	Occupational asthma; specific inhalation challenges were positive in 29 subjects.	(Cartier et al., 1989)
Case report of two subjects showing respiratory symptoms	Not specified	MDI	Occupational asthma	(Malo et al., 1989)
Group-based report on 63 workers with a diagnosis of probable isocyanate-induced asthma	Manufacture of TDI, manufacture of foam, manufacture of refrigerators	TDI	TDI-induced asthma in 30/63 workers	(Banks et al., 1989)
Case report of one subject complaining of nocturnal dyspnoea and dry cough	Paint processing plant	TDI	Hypersensitivity pneumonitis due to isocyanates	(Nozawa et al., 1989)
Case report of one patient with symptoms of non-cardiac chest pain probably secondary to pleuritis	Worker manufacturing award placques with a polyurethane coating resin containing MDI	MDI	Isocyanate-induced asthma	(Sales and Kennedy, 1990) and
Case report of six workers with respiratory complaints	Production of polyurethane foam; #1, 2, 3, 5: Workers manufacturing polyurethane foam #4: Research technician #6: Worker in the shipping department; Later all six worked in areas with negligible/no exposure to TDI	TDI	TDI-induced occupational asthma	(Banks et al., 1990)
Case report of 13 workers with respiratory symptoms consistent with asthma	Manufacture of waferboards; workers performing routine (i.e. waxing of former conveyor belt) and non-routine (unplugging jammed conveyors, repairs, adjustments) maintenance tasks	MDI	Occupational asthma (12 cases) and hypersensitivity pneumonitis (1 case)	(Reh and Lushniak, 1984)
Case report of one patient with, *inter alia*, bilateral pleuritic chest pain and haemoptysis	Spray-painter spraying isocyanate-containing paint onto warm metal	HDI, another isocyanate (possibly TDI)	Haemorrhagic pneumonitis	(Patterson et al., 1990)
Evaluation of the morphologic basis of the different outcomes of TDI asthma after quitting occupational exposure in ten patients with TDI asthma	Not specified	TDI	Asthma	(Paggiaro et al., 1990)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Case report of one patient having bronchospasms after burning polyurethane packs and an immediate asthmatic reaction while working with polyurethane foam.	Task at work:Burning polyurethane packsTask at home: Insulating a window/drilling dry polyurethane foamTasks with unspecified location: Painting cars with isocyanate-containing paints	MDI, TDI	Immediate bronchial hyperreactivity	(Dietemann-Molard et al., 1991)
Study reassessing temporal patterns of bronchial obstruction after exposure to diisocyanates in 23 subjects that were referred for investigation of occupational asthma and underwent specific inhalation challenges with positive results	Six foam industry workers, ten spray painters, seven employees from various industries (plastics, foundries)	HDI, MDI, TDI	Occupational asthma	(Perrin et al., 1991)
Study of blood parameters in ten subjects, previously shown to develop a dual or late asthmatic reaction after inhaling TDI	Not specified	TDI	Occupational asthma	(Finotto et al., 1991)
Evaluation of 23 employees complaining about work-related respiratory symptoms	Paint mixers and spray-painters	TDI	Asthma in 3/23 patients	(Park et al., 1992)
Case report of two workers with asthma	Wood-roof maintenance workers brushing/rolling lacquers/varnishes containing TDI	TDI	Occupational asthma	(Vandenplas et al., 1992a)
Case-control study of activated T-lymphocytes and eosinophils in the bronchial mucosa of patients with isocyanate-induced asthma; nine occupationally sensitised subjects and twelve healthy non-atopic control subjects were tested.	Not specified	MDI, TDI	Occupational asthma	(Bentley et al., 1992)
Case study of a man with dry cough and exertional dyspnoea	Handling spray-paint containing isocyanates	TDI, MDI	Hypersensitivity pneumonitis	(Akimoto et al., 1992)
Cross-sectional study in 216 coal-miners exposed to MDI showing symptoms of work-related shortness to breath	Coal miners working in rock consolidation with MDI	MDI	Specific bronchial hyperresponsiveness to MDI (4), isocyanate asthma (2)	(Lenaerts-Langanke, 1992)
Evaluation of closed-circuit methodology for inhalation challenge test with isocyanates in 20 consecutive workers suspected of having isocyanate-induced asthma	Not specified	HDI, MDI, TDI	Occupational asthma in 6/20 workers	(Vandenplas et al., 1992b)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Specific inhalation challenge study in workers with possible occupational asthma	Not specified Workers exposed to spray paints	HDI	Occupational asthma in 10/20 workers	(Vandenplas et al., 1993a)
Inhalation challenge study in workers complaining of respiratory and general symptoms related to workplace exposure	Manufacture of woodboard chips with MDI-based resin #1: Maintenance mechanic #2: Production line welder #3: Quality control laboratory #4: Electrician #5: Industrial mechanic #6: Production supervisor #7: Cleaning #8: Casual	MDI	Hypersensitivity pneumonitis	(Vandenplas et al., 1993b)
Examination of seven subjects with occupational asthma induced by TDI or MDI and three control subjects never exposed to isocyanates	Not specified	MDI, TDI	Occupational asthma	(Calcagni et al., 1993)
Patient claiming compensation for bronchial asthma	Surface worker in a coal mine involved in polyurethane rock consolidation	MDI	Occupational asthma	(Nemery and Lenaerts, 1993)
Case-control study of sputum eosinophilia after asthmatic responses induced by isocyanates in 9 subjects with occupational asthma induced by MDI or TDI and four control subjects	Not specified	MDI, TDI	Occupational asthma	(Maestrelli et al., 1994a)
Study examining CD8 T-cell clones in bronchial mucosa of two patients with asthma induced by TDI	Use of polyurethane paint	TDI	Occupational asthma	(Maestrelli et al., 1994b)
Case report of 14 patients suspected of isocyanate-induced hypersensitivity pneumonitis.	#1, 3, 10, 12, 14: Foam production #2, 8, 9: Paint spraying (#4: Plastic welding) #5, 11: Adhesive application #6, 7, 13: Injection molding	HDI. MDI, TDI, HDI, (TDA/TIPHP in #4)	Hypersensitivity pneumonitis	(Baur, 1995)
Study on the outcome of specific bronchial responsiveness to occupational agents after removal from exposure in 15 subjects with occupational asthma	Not specified	HDI, MDI, TDI	Occupational asthma	(Lemière et al., 1996)
Case report of one subject with occupational asthma	Steel foundry; mold and core processing with use of resins containing MDI	MDI	Occupational asthma (1986) followed by fatal asthma attack (1992)	(Carino et al., 1997)
Case report of one subject with breathing difficulties	Carpenter/glueing wood onto aluminium sheets	MDI	Asthma and contact urticaria	(Kanerva et al., 1999)

Subject of the study	Occupation/task	Agent(s)	Diagnosed disease/effects	Reference
Inhalation challenge study in 24 symptomatic subjects	Not specified	HDI, MDI, TDI	Occupational asthma	(Malo et al., 1999)
Analysis of specifig IgG response to isocyanates in 13 subjects with respiratory reactions	Not specified	HDI. MDI, TDI	Occupational asthma (12), hypersensitivity pneumonitis (1)	(Aul et al., 1999)
Case report of one worker with respiratory symptoms, who was exposed for three years without developing sensitisation. Probably a single high dose after an accidental spill represented the trigger for sensitisation	Toy manufacture; spray painter/spray painting of polyurethance foam balls with a paint containing MDI	MDI	Occupational asthma	(Perfetti et al., 2003)
Case report of a woman with breathing difficultie; symptoms started after a peak exposure (heavy and prolonged contact with a glue).	Manufacture of plastic components for the car industry using a two-component polyurethane glue	MDI	Occupational sensitisation to MDI causing contact urticaria and asthma simultaneously	(Valks et al., 2003)
Case report of one man complaining about respiratory symptoms	Handling of spray-paint containing isocyanate	MDI	Combined hypersensitivity pneumonitis and bronchial asthma	(Matsushima et al., 2003)
Case report of one patient with respiratory symptoms	Hospital nurse working with MDI-containing synthetic plaster casts	MDI	Occupational asthma	(Donnelly et al., 2004)
Case report of one man who reported coughing and fever	Breaking up a large refrigerator containing MDI	MDI	Hypersensitivity pneumonitis with acute respiratory distress syndrome	(Morimatsu et al., 2004)
Re-examination of 25 subjects diagnosed with occupational asthma after long-term removal from exposure	Spray-painting using polyurethane varnishes	TDI	Occupational asthma; re-examination of subjects with occupational asthma after 58 ± 7 months following removal from exposure. Seven were still reactors, 18 had lost reactivity.	(Pisati et al., 2007)
Case report of one subject complaining of breathing difficulties	Mixing polyurethane glues for the manufacture of adhesives	MDI	Asthma and urticaria (concomitant type I and type IV sensitivities to MDI)	(Stingeni et al., 2008)
Follow-up study in 17 patients diagnosed with diisocyanate-induced asthma after cessation of exposure	Not specified	HDI, MDI, TDI	Diisocyanate-induced asthma	(Piirilä et al., 2008)
Case report of one patient with an acute respiratory event	Paint quality controller (laboratory)	HDI	Occupational extrinsic allergic alveolitis; life-threatening allergic reaction	(Bieler et al., 2011)

Table 2 shows the results from studies regarding the annual incidence of TDI-related occupational asthma cases as reviewed by (Ott, 2002).

Table 2: Data taken from Tables II and III in (Ott, 2002)

Study	Time period	Annual incidence of TDI-induced occupational asthma [%]	TDI concentration [ppb]	Exposure sampling
TDI production units				
(Adams, 1975)	1961 - 1970	5.6	1962 - 1964: 58-72% of samples > 20 1965 - 1966: 4-21% of samples > 20 1967 - 1970: 1-2% of samples > 20	Area samples
(Porter et al., 1975)	1956 - 1959	1.6	1956 - 1957: 60 (mean area conc.)	Area samples
	1960 - 1969	0.8	1960 - 1969: steady decline in area conc.	
	1970 - 1974	0.3	1974: < 4 (mean area conc.)	
(Weill et al., 1981)	1973 - 1978	1.0	1.6 - 6.8 (TWA; range by job) (STC > 20, 5-11% of time in moderate to high exposure jobs)	Area samples 1973-75 Personal samples 1975-78
(Ott et al., 2000)	1967 - 1979	1.8	3.4-10.1 (TWA; range by job)	Area samples 1967-75 Personal samples 1976-96
	1980 - 1996	0.7	0.3-2.7 (TWA; range by job) (STC > 20, 0.5-0.9 times/shift in moderate to high-exposure jobs)	
PU foam production facilities				
(Woodbury, 1956)	1954 - 1955	5	Multiple TDI spill episodes described in 18-month period	No sampling data
(Williamson, 1964)	1962 - 1963	> 2.7	Samples mostly < 20 (up to 200 detected during spills)	Area samples
(Bugler et al., 1991)	1981 - 1986	0.8	0.9-2.6 (TWA; range by job) 22% of 8-h samples with short-term conc. > 20 and 10% > 40	Personal samples
(Jones et al., 1992)	1982 - 1986	0.7	1.4-4.5 (TWA; range by job) (STC > 20, 3% of time in production and 0.1% of time in finishing jobs)	Personal samples

1.1.1.2 Longitudinal studies

The available longitudinal studies are summarised in Table 3.

Table 3: Longitudinal studies on occupational asthma related to exposure to HDI, MDI, and/or TDI

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Adams, 1975)	Prospective cohort study (nine years), two plants 565 subjects employed for some period between 1961 to 1972 A) Comparison of respiratory symptoms in TDI plant workers (n = 76) with control workers (n = 76) in another plant B) Lung function in healthy workers (n = 180) C) Long-term effects in men removed due to symptoms without exposure to TDI since two to 11 years (n = 46) compared to age-matched control group (n = 46) D) Lung function in men removed due to symptoms and without exposure to TDI since two to 11 years (n = 61)	TDI Manufacture	Area samples taken at points in the plant where free TDI might have been expected (ca. 250 measurements a week; Marcali method, (Marcali, 1957)) Samples > 20 ppb: 1962-64: 58–72% 1965-66: 4–21% 1967-70: 1-2%	A) **Respiratory symptoms** (questionnaire): No significant difference in symptoms between men working in TDI plant and controls, with the exception of higher frequency of wheezing in controls. B) **Lung function**: Duration of exposure had no effect on FEV_1 or FVC in the regression analysis. C) **Respiratory symptoms** (questionnaire): Prevalence of symptoms in TDI-sensitised men significantly higher than in controls → persistence of symptoms D) **Lung function**: FEV_1 and FVC smaller than predicted by equation obtained from a control group: FEV_1 -267 mL, FVC -269 mL	Reviewed in (Ott, 2002) Method of analysis did not calculate individual decline in lung function. Regression analysis included duration of exposure, but no exposure level Area measurements Lung function measurements in the afternoon Only healthy workers included Smoking not included in regression analysis

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Butcher et al., 1977)	Prospective cohort, 2.5 years Visits: April 1973 (before TDI production), November 1973 (after production had started), every six months thereafter Initially n = 166 Study in TDI-sensitive persons (specific and unspecific challenge)	TDI Manufacture	Area sampling (1973): frequent excursions of 8h-TWA value of 5 ppb; many above 20 ppb Personal monitoring (1975) Frequent and large discrep-ancies between simultaneously measured area and personal exposure levels Four groups: 1) Mainly in TDI area: n = 77 2) Intermittently in TDI area: n = 36 3) Comparison group: n = 53 4) Workers transferred from control group to exposure group after production had begun (added later)	**Lung function** changes (n = 102): Mean values of FVC and FEV_1 increased in all groups. Other lung function parameters decreased slightly (n. s. different from zero or predicted). Paradoxical differences for lung volumes and diffusion capacity (greater declines in the groups with higher exposure). No exposure-related excess decline in lung function determined. **Respiratory symptoms** (questionnaire administered by interviewers): No significant increase in prevalence of bronchitis, atopic disorders, upper respiratory symptoms from April 1973 to October 1975. Significant proportion of exposed workers (26 of 89) reported onset of lower respiratory symptoms after beginning work in TDI areas (due to symptom development in non-smokers). **Inhalation challenge** with TDI: Nine out of 13 workers had an adverse bronchial response (immediate type, late type or dual type). Some reacted at 5 ppb, some to a higher concentration only.	Attrition rate = 7.2% Two workers had left the study by October 1975 after developing reactivity to TDI. No quantitative exposure es-timation for the four exposure categories Smoking not considered in analysis of change in lung function

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Wegman et al., 1977)	Follow-up of (Wegman et al., 1974) 1972: n = 112 1974: n = 63 (available for re-survey); n = 57 with personal exposure levels	TDI PU cushion manufacture	118 area samples + 14 personal samples taken during study period to characterise 20 work stations Marcali method (Marcali, 1957) Each individual was classed according to his or her usual work station Three exposure groups (ppm): ≤ 0.0015 (n = 20) 0.0020 – 0.0030 (n = 17) ≥ 0.0035 (n = 20)	**Lung function** (because of acute effect seen on Monday: Monday morning following three-day weekend): Dose-response relationship for two-year change in FEV_1 (-12/-85/-205 mL from low to high exposure groups). Only those in lowest exposure group showed normal declines in $FEV_{1.}$ Those in highest group had three- to fourfold higher FEV_1 declines than expected (103 mL/year). Significant association between acute and chronic decrement in $FEV_{1.}$ **Respiratory symptoms** (questionnaire): Prevalence of cough and phlegm increased with increase in exposure. Wheezing and dyspnea not associated with exposure.	High attrition rate Followed up: (Wegman et al., 1982) Possible confounding variab-les explored: Age, months employed, smoking habits, variables related to lung size. Authors report that none of those was able to explain the differences.
(Diem et al., 1982)	Five-year prospective (9 surveys) First survey in 1973 (5 months before start of production) Initially: n = 168 After 5 surveys: n = 274 (males) Median follow-up time for n = 223 men who met inclusion criteria of spirometric data 4.1 years (1 – 5.5)	TDI manufacture	2093 personal samples from 143 workers representing all job categories 8 h TWA from 0.1 ppb - 25 ppb, geometric mean 2.00 ppb Average exposure: Three TWA exposure job categories: Geometric mean in ppb (time per shift < 20 ppb): Low: 0.02 (1.3 min) Medium: 2.0 (8.6 min) High: 4.5 (28.2 min)	**Lung function** (spirometry, annual change): Decrease in FEV, %FEV and FEF_{25-75} was significantly larger in the high cumulative exposure category than in the low category (adjusted for pack-years of smoking). No association of the other lung function annual changes with exposure. A more detailed analysis of FEV_1 and FEF_{25-75} in six categories of cumulative TDI exposure and smoking showed a significant effect of TDI exposure in never smokers only and a significant	No unexposed group "The present data do not identify a specific exposure below which no effect upon FEV_1 annul decline will occur. However, they do suggest that the NIOSH-recommended standard of a 5 ppb 8-h time-weighted average and a 20 ppb 10-min short-term exposure limit is reasonable."

Reference	**Study design and subjects**	**Isocyanate and use**	**Exposure**	**Results**	**Remarks**
(Diem et al., 1982), ctd.			Cumulative exposure calculated from number of months spent in each of the three TWA exposure categories and their respective geometric means. Workers were divided into two groups using a division point of 68.2 ppb-months (= 1.1 ppb x 62 months). Low exposure group n = 149, high n = 74. Working time spent > 5 ppb: 2% in low exposure group, 15% in high exposure group. Peak exposure categories: Division point 0.19 months > 20 ppb	effect of smoking in the low exposure group only. → effects not additive Effects similar for six categories of TDI peak exposure and smoking with the exception that a significant exposure effect was also found in current smokers → higher TDI exposure seems to mask smoking effect → peak exposure analysis suggests additive effect (lacking in cumulative exposure analysis) **Respiratory symptoms** (questionnaire): No significant correlation in increase in prevalence from initial to final interview and exposure to TDI.	Low cumulative exposure group was older and initially had higher prevalence of respiratory symptoms than high exposure group → possible underestimation of excess decline in lung function due to TDI 75% of the low exposure group had follow-up time > 2.5 years and 99% of the higher exposure group Atopy, race and smoking were considered Age and FEV_1 level were considered in the more detailed analysis of FEV_1 and FEF_{25-75}
(Musk et al., 1982)	Five-year follow-up n = 259 from three sites were examined in 1971; one of the sites closed in 1972 and there was high worker turnover; 107 subjects were available for re-examination in 1976	MDI and TDI for the manu-facture of PU automobile components	2573 environmental samples were collected by plant personnel in the breathing zone of subjects pouring urethane plastic (exposure in areas with the highest exposures were measured) During lung function survey further measurements were made by plant personnel and study personnel at selected sites with highest TDI and MDI concentrations Marcali method (Marcali, 1957)	**Lung function** (spirometry (FEV_1, FVC); change over 5 years/change over the course of a day/change between before and after two weeks of vacation): Mean annual decrement in FEV_1 of 0.02 L was interpreted as being only age-related No significant acute change in FEV_1 over the course of a day before or after vacation reported After two weeks of vacation FEV_1 was increased in those who had taken the vacation (n = 49, n. s.) and was decreased in those who had worked (n = 31, n.s.).	Uncertainties in exposure assessment and spirometry Smoking, age, height, sex were considered in the regression analysis of FEV_1. Healthy worker survivor effect (although it is reported that subjects who left had similar lung functions to the remaining subjects, it seems possible that workers left due to earlier symptoms of sensitisation).

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Musk et al., 1982), ctd.			All environmental measurements made over the 5 years together with the occupational history of the subjects determined the exposure category (No exposure/MDI/TDI/MDI and TDI). 90% of all measurements of TDI taken over the four years prior to the follow-up study were < 5 ppb (plant 1) and < 4 ppb (plant 2) Geometric mean TDI concentration: 1.5 ppb (plant 1) and 1 ppb (plant 2) MDI levels tended to be lower than TDI levels	Exposure category did not affect daily change in FEV_1/pre- to postvacation change in FEV_1/five-year change in FEV_1. **Respiratory symptoms** (questionnaire): No association between exposure to isocyanates and bronchitis or dyspnea found No acute exposure-related symptoms reported	
(Wegman et al., 1982)	Four-year follow up (Wegman et al., 1974; Wegman et al., 1977) 1972: n = 111 1974: n = 63 1976: n = 48 (all those who were still at work in 1976) → n = 37 with exposure history and acceptable spirograms On all three occasions workers were examined before work and as many as possible six to ten hours later.	TDI Automobile seat cushion manufacture	Environmental sampling at selected work sites on the same day as lung function was measured. Additional sampling during the first two years of the study. Personal sampling in production area, area samples in warehouse and nonproduction sites. Marcali method (Marcali, 1957) Occupational histories taken from personnel records	**Lung function**: Acute change in FEV_1 (during work shift) observed at the beginning of the study was weakly associated with long-term change in FEV_1. Chronic change in FEV_1 (over four years): Mean exposure to TDI was the best predictor of four-year change in FEV_1 in a stepwise regression model. Change in FEV_1 increased with exposure and was significantly different between the exposure groups.	Uncertainties in exposure assessment High attrition rate Lung function decline evaluated from 3 occasions only

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Wegman et al., 1982), ctd.			Cumulative exposure of each worker calculated and from this the usual exposure level. Three exposure groups: Low (< 2.0 ppb) Medium (2.0-3.4 ppb) High (> 3.5 ppb)	Decline in FEV_1 in high exposure group (60 mL/year) was higher than annual decline observed in other studies of normal populations (32-47 mL). **Respiratory symptoms** (questionnaire; upper respiratory tract symptoms: sneezing, sinus trouble or postnasal drip, hay fever; lower respiratory tract symptoms: coughing, wheezing, shortness of breath): Prevalence of respiratory symptoms was unrelated to exposure category.	
(Omae, 1984)	Two-year follow up Four TDI-producing plants, two research laboratories 1980: n = 106 male exposed workers n = 39 male controls (office workers) 1982 (one plant had closed): n = 64 workers (follow-up rate 60%) n = 21 controls (follow-up rate 62%)	TDI Manufacture; research laboratory	Mean duration of TDI exposure: 9.0 years (subjects in 1980) 11.2 years (subjects in 1982) Personal paper tape monitor (gives continuous profile; n = 161 samples in 1980, 106 in 1982) Means of individual TWA: 0.7 ppb (1980) 1 ppb (1982) Short-term exposure ≥ 20 ppb in 9.3% (1980) and 1.9% (1982) of collected samples	**Lung function** (Maximum expiratory flow volume curve, respiratory impedance): Eight workers with asthmatic reactions, shortly after having begun work with TDI. Percentage of predicted values significantly less than 100% in some of the expiratory flow parameters. No significant differences in lung function between the exposed workers and the referents. Change in lung function over the day (1980; 68 TDI workers + 31 controls): No meaningful daily changes in lung function in either group. Change in lung function over two years: When adjusted for aging, no remarkable intra-individual two-year decreeses in lung function parameters in both groups and no significant difference between the groups.	High loss to follow-up Co-exposures: TDI plant workers: occasionally various irritants such as phosgene, chlorine, nitric acid, sulfuric acid; Research laboratory workers: irritative amines, organic tin compounds , MDI, HDI during experimental mold foaming Effects of age, physical factors and smoking on lung function considered in analysis Survival worker effect considered to be small by the authors Hyperreactive persons to TDI may have already been transferred out of TDI sections

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Omae, 1984), ctd.				No difference in the two-year decrement between the workers with asthmatic reactions and the other TDI workers. **Symptoms** (interviewed by means of a questionnaire): No significant differences in prevalence of respiratory symptoms between exposed workers and reference. Significantly higher prevalence of throat and eye irritation in exposed workers than in reference. May be due to peak exposures to TDI or other irritants (phosgene).	
(Gee and Morgan, 1985)	Ten-year follow-up (includes significant proportion of subjects included in (Musk et al., 1982)Musk et al. 1982) Examinations in 1971 and in 1981 n = 68 exposed n = 12 controls n = 65 subjects with pre- and post-shift measurement n = 42 studied in 1971 and 1981	MDI and TDI Manufacture of fittings, seat covers, other fixtures used in the interior of cars	Routine area and some individual sampling had been carried out monthly or more frequently Mean annual concentrations between 1973 and 1980 for TDI: 1- 5 ppb Mean annual concentrations between 1975 and 1981 for MDI: 1- 5 ppb	**Lung function** (compared to predicted values): Three subjects had impaired lung function (two exposed, one control). Lung function of subjects studied previously had mean FVC and mean FEV_1 > 100% of the predicted values. Control group of one plant had a significantly lower percentage of the predicted FVC and FEV_1 than the exposed group. No other significant difference between any of the groups. Lung function (change over shift): Change not higher than 10% in any subject. No comparison between controls and exposed.	Mean annual exposure values on factory level only Uncertainties in spirometry data (no reproducibility, leak in spirometer possible in 1971; learning effect from pre- to post-shift measurements) Results on annual decline in lung function seen as “not realistic” (small increase in FVC, small decrease in FEV_1).

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Gee and Morgan, 1985), ctd.				Mean shift change in FEV_1 was -57 mL in exposed and +69 mL in controls in one plant and -23 and -80 mL in the other plant, respectively.	
(Musk et al., 1985)	Re-analysis of the data from (Musk et al., 1982)				The spirograms performed 1971 in the study by (Musk et al., 1982) were criticised ("inadequate", "lack of reproducibility", "leak in the spirometer"). (Musk et al., 1985) found the original conclusions valid.
(Pham et al., 1988)	Five-year follow up 1976: n = 318 workers (104 women) 1981: n = 156 (45 women) Two factories producing PU foam Follow up of Pham et al. 1978	Mainly MDI Production of PU foam	Isocyanate concentration: 1976: < 20 ppb 1981: ≤ 5 ppb 1976: Group I (n = 83): unexposed Group II (n = 117): indirectly exposed Group III (n = 118) directly exposed 1981: Only results for men reported for the longitudinal analysis. Group A (n = 45): unexposed at both studies Group B (n = 24): undirectly exposed at both studies Group C (n = 30): directly exposed at both studies Group D (n = 15): exposed in 1976, but removed in 1981	**Lung function** (flow volume curve, single breath CO diffusion test (D_{LCO})): Ventilatory function and lung transfer factors significantly impaired in male exposed workers compared to group I. Only in the subgroup of workers exposed for more than 5 years. Decline of ventilatory function variables not significantly different between the groups. Significant larger loss of D_{LCO} in subjects with persisting exposure (group C) compared to reference group. Results returned to normal for the subjects no longer exposed (group D). **Respiratory symptoms** (question-naire): Increased prevalence of asthma in group II men and group III women and of chronic bronchitis in both sexes. Number of workers with asthma or chronic bronchitis increased over the five years, but this was not limited to the exposed group.	High loss to follow up (only half of the initial cohort still active after 5 years) Rare information on exposure In females, the proportion of smokers was the same in groups I – II. In males, there were slightly (n.s.) more smokers in groups II and III. Co-exposure to other isocyanates? ("mainly MDI")

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Tornling et al., 1990)	Six-year follow-up (initial study: (Alexandersson et al., 1987)) 1978: 46 male car painters and 142 male controls (car platers and mechanics) randomly chosen from 14 garages in Stockholm Reinvestigation in 1984: Participation rate 78% for car painters and 81% for controls n = 36 car painters n = 115 controls	HDI monomer (and HDI biuret trimer) Car painting	Individual exposure assessments by industrial hygienist (interview about working routines, respirator use, hygienic standards). Exposure measurements at seven representative shops 98 samples inside and outside the respirator Individual exposure was calculated from workplace data, proportion of work tasks, use of respirators. 18 peak exposure measurements (sampling time < 3 min) Calculated TWA exposure: HDI: 0.0015 mg/m^3 (HDI-BT: 0.09 mg/m^3, frequently peak exposures > 0.2 mg/m^3) Calculated yearly number of peak exposure situations up to 6000 for each car painter No close correlation between exposure peaks and mean exposure	Decline in **lung function** over six years (1978: Monday morning values were used; 1984: Workers were examined during the first three hours of a working day): Smoking and ex-smoking car painters had significantly larger lung function decrease compared with respective controls. Non-smoking car painters displayed no faster deterioration in lung function than corresponding controls. (Decrease in FVC correlated significantly with number of HDI-BT exposure peaks, but not with mean exposure.) **IgG and IgE**, specific IgE in car painters: No significant differences in Ig levels between car painters and controls. No specific IgE found. **Symptoms**: Car painters reported significantly higher frequency of wheezing than the controls. Differences for other symptoms n.s.	Participation rate at follow-up 78% among car painters and 81% among controls. Selection bias (drop-outs may have quitted job because of respiratory symptoms, one asthma case known) Smoking not quantified

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Jones et al., 1992)	Cross-sectional, follow up Two plants n = 394 at the start of the study, through the fourth examination n = 435 had ever worked in one of the plants	TDI Production of flexible PU foam products	258 workers wore monitors on 507 shifts resulting in 4845 12-min samples: 9% > 5ppb 1% > 20 ppb TDI concentrations were assigned to groups of jobs. Information on the number of months spent in each exposure grouping was taken from personal records. Mean by plant and job area ranged from 1.17 to 4.47 ppb. Exposure measures: Cumulative exposure from hire to first study examination; cumulative exposure from hire to the end of study; cumulative exposure during the study period; length of time exposed to concentrations > 5 and 20 ppb	**Lung function** (spirometry, standing position, nose clips): Significant adverse effect of cumulative TDI exposure on initial level of FVC and FEV1 in current smokers. TDI exposure had no significant effect on lung function decline. **Respiratory symptoms** (questionnaire administered by trained interviewers): Chronic bronchitis more prevalent among those with higher cumulative exposure (controlled for smoking, age, sex). Metacholine challenge (n = 303): Metacholine responsiveness in 22% of tested workers. Skin prick test with common inhalant allergens Total IgE, RAS	Co-exposure to different amines and other substances in foam production Healthy worker effect (predicted values) Differential misclassification of exposure (large number of samples < LOD)

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Omae et al., 1992)	Four-year follow up (cross-sectional results see (Omae et al., 1992)) Cross-sectional: 1981 Follow-up visits: 1983 and 1985 Japan: 57 PU foam workers (follow-up rate 66%; two excluded) 24 reference workers (follow-up rate 61%; three excluded)	TDI PU foam manufacture	Personal paper-tape monitors (n = 59 samples in 1981, 48 in 1983 and 52 in 1985) Group L (low exposure with little variation), n = 28, 17.4 years in the PU foam factories (mean), TWA (mean, max) 0.1 ppb, 1 ppb; Peak exposure level < 1 ppb Group H (exposed workers), n = 29, 16.5 years in the PU foam factories (mean), TWA (mean, max) 5.7 ppb, 30 ppb; Peak exposure level 3-80 ppb Two subgroups of group H: Group H1 (high short-term exposures), n = 15, 13.8 years in the PU foam factories (mean), TWA (mean, max) 8.2 ppb, 30 ppb; Peak exposure level 30-80 ppb Group H2, n = 14, 19.4 years in the PU foam factories (mean), TWA (mean, max) 1.7 ppb, 4 ppb; Peak exposure level 3-14 ppb	**Lung function** (Flow-volume indices in 1981; Average annual loss of the indices during 1981-1985 (forced expiratory flow-volume test at follow-ups; slope of the regression equation for every subject)): No "noteworthy" differences in pulmonary function indices and average annual losses between groups H, L, reference. Group H1: Significantly larger average annual lung function losses (%MMF, $\%FEV_1$, $\%MEF_{25}$) than expected. Significantly larger average annual losses in some obstructive pulmonary function indices than in group L or reference group.	No individual exposure estimates No significant differences between group H1 and H2 (as suggested in the abstract) Workers in slab-type factories intermittently exposed to relatively high levels of TDI and concurrent other chemical gases/aerosol → group H divided into two subgroups Smoking rate significantly lower in group H than in group L and reference group Comparison of average annual losses of smokers and non-smokers in the 4 groups showed similar trends. Higher losses in smokers than non-smokers. Based on a comparison between lung function of followed-up and lost workers, survival-worker effect was evaluated to be small.

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Dahlqvist et al., 1995)	Re-analysis of data from (Tornling et al., 1990) and (Alexandersson et al., 1987) Evaluation if lung function decrease within the week is a marker of vulnerability of further decrement in lung function Six-year follow up, two study occasions Original group of workers were randomly chosen from 14 garages in Stockholm, 28 car painters participated in all three spirometric examinations, only those 20 were chosen who had been working during the entire six years period	HDI Monomer (and biuret trimer) Car painters working with polyurethane paints	Individual exposure assessments by industrial hygienist (interview about working routines, respirator use, hygienic standards). 81 exposure measurements for three tasks in 25 spray-painting chambers. Peak exposure measurements were performed (sampling time < 3 min) TWA between 1978 and 1984 for the workers studied: HDI: 0.0014 mg/m^3 (HDI-BT: 0.09 mg/m^3)	**Lung function** (1978: spirometry on Monday before work after two days of no exposure and on Friday; 1984: spirometry during the first three hours of a working day) Changes in FEV_1 and FVC within the week were dichotomised. Ten workers had a decrease in FVC within the week. Ten workers had a decrease in FEV_1 within the week. Car painters in the initial study who showed a decrease of FVC within the week in 1978 had a significantly greater decline in FVC from 1978 to 1984 than car painters who did not (adjusted for smoking). Significant correlation between changes within the week and six years decline in FVC. Decline in FVC was not significantly correlated with the mean exposure to HDI (or HDI-BT) estimated during the entire follow up. (Six year decline in FVC was correlated to the yearly number of peak exposures to HDI-BT.) **Respiratory symptoms** reported (for example 3/10 workers with change in FVC within the week in 1984 had cough, dyspnoea, and/or wheeze).	Uncertainties in exposure assessment (Current smokers on average had a higher yearly number of peak exposures to HDI-BT than the smokers as a whole (previous and current)..May indicate less use of protective equipment by smokers.) Smoking not quantified

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Akbar-Khanzadeh and Rivas, 1996)	1) Cross-sectional (daily, weekly changes) 2) Longitudinal (2.5-year follow up) 1) 16 Urethane mold operators 19 Controls (final assembly department, office area) 2) Oct 1989 – March 1992: 65 exposed to diisocyanates and solvents 40 exposed to solvents 68 controls (office, assembly, hardware department)	HDI monomer (and polyisocyan-ate), combined with organic solvents (MDI) Encapsulated automobile glass plant	1) HDI monomer, HDI polyiso-cyanate, volatile organic compounds Personal and area samples HDI: 92% < LOD (set to 50% of LOD); mean concentration (personal, area): 1.55 ppb (n = 6), 0.65 ppb (n = 3) (HDI polyisocyanate: 75% < LOD; mean concentration (personal, area): 0.09 mg/m^3 (n = 6), 0.02 mg/m^3 (n = 3)) 2) Mean concentration: HDI 1 ppb (n = 8 samples) (HDI polyisocyanate 0.29 mg/m^3 (n = 5 samples)) MDI 0.45 ppb (n = 7 samples)	1) **Lung function** (spirometry on Monday and Friday before and after shift): No significant differences between exposed and control group No significant reduction in lung function during workshift or during week in the exposed group compared to the control group. Some findings in subgroups by sex. **Respiratory symptoms** (questionnaire): Some symptoms more prevalent in control group (n. s. or not tested?). 2) **Lung function** (spirometry before the shift): Significant decrease in lung function parameters in isocyanate/solvent-exposed group. Significant differences in lung function change (FEV1 and FVC) among groups **Respiratory symptoms** (questionnaire): Proportion of subjects who developed respiratory symptoms in the isocyanate-exposed group was not significantly greater than that of the non-exposed group.	No individual exposure estimates Very small number of air samples Control group appropriate? 1) HDI in control area 0.67 ppb Co-exposure Smoking was significantly more prevalent in the exposed group 2) Co-exposure Controls had no occupational exposure “between the two tests”

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Clark et al., 1998)	5 years longitudinal UK 780 workers in 12 factories (623 original + 157 naïve workers)	TDI Manufacture of PU foam	Personal monitoring (2294 measurements) for 100 job categories. Cumulative exposure between first and last lung function measurement was calculated for each subject based on job histories. 8 h TWA exposure limit of 5.8 ppb (46 ppbh for an 8 h working day) was exceeded on 107 (4.7%) occasions. Five of the 780 subjects (0.6%) had a mean daily exposure exceeding the limit value. Peak exposure limit value of 20 ppb was exceeded in 500 (19%) samples. 8.8% of the peak measurements > 40 ppb Exposed group (n = 521): Manufacture of PU foam or handling freshly manufactured products; mean daily exposure 9.6 ppbh (1.2 ppb 8 h TWA) Handling group (n =123): Handling cold PU products Low-exposure group (n =136): shop floor and office workers	Longitudinal decline in **lung function** (spirometry; three or more measurements): No significant effect of TDI on annual lung function change. For the naïve population, regression analysis showed a significant effect of mean daily exposure on annual changes of FEV_1 and FVC. Due to irritant effect? **Respiratory symptoms** (questionnaire): Increase in respiratory symptoms in exposed group and handling group, significant for wheezing. 24 cases of respiratory sensitisation were identified during the study.	Followed up by Clark et al. 2003 High attrition rate (47%) Leavers reported excess breathlessness and wheeze compared to non-leavers of the total population. Linear regression considered sex, group, age, age^2, smoking, mean daily exposure, peak exposure, pre-study exposure.

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Hathaway et al., 1999)	Nine-year follow-up Production began in 1988, follow up through 1997 n = 43 "potential cases" and n = 42 "potential controls" of another unit at the same plant n = 32 matched pairs (by smoking, sex, age and by race and height if multiple possibilities were available)	HDI Production of HDI biuret and trimer from monomer	Average number of years of potential exposure: 8.4 Area and personal sampling (different methods and equipment over time) Exposure when not wearing respiratory protection was considered 1992-1995 (personal monitoring): average (range): TWA during work not requiring respiratory protection in the unit (1 – 4 hours/day): 0.5 ppb (0.0 – 2.0 ppb); calculated as 8h-TWA: 0.13 ppb Highest daily peak exposure: 2.9 ppb (1.0 – 10.0) Exposure before 1992 believed to be somewhat higher (no quantification)	**Lung function** (as part of annual evaluation of workers): Average number of available tests for calculating slope: 7.8 (exposed) and 8.2 (controls). No significant difference in annual change of lung function (slopes) between exposed and control group. By smoking status, the results show more variation. Results seen as being within the range of lung function declines reported in other studies.	Exposure not measured on individual level Smoking not quantified Height and race only partially controlled Co-exposure in control group reported (depending on work area): cerium and neodymium oxides, nitric acid, ammonia, kerosene, tributyl phosphate Qualitative information on potential drop outs: low turnover rate, few transfers between the units, subject attrition not been a problem
(Ott et al., 2000)	Historic cohort study using medical records and exposure records from 1967 to 1997 313 employees ever assigned to the TDI production unit for ≥ 3 months; 158 reference employees;	TDI manufacturing	Duration of TDI unit assignments: 5.7 years (average, men) 4.7 years (average, women) 3 months to 30 years (range) 1967 (area sampling): < 10 ppb in most areas and 25 ppb in the residue handling area	**Occupational asthma**: Case identification was based on site physician. One episode of asthma-like symptoms was not enough to be an OA case. 19 asthma cases presumed to be due to TDI, 9 skin allergies, 1 case of asthma and skin disease; Yearly incidence: 19 cases in 1779 work years = 1.1%; before 1980: 1.8%; since 1980: 0.7%	Long follow-up time Exposure concentration linked to the asthma incidence not clear. (Ott et al., 2003) report for this study an exposure of 0.3 – 2.7 ppb (TWA; range by job) since 1980, assigning this to a yearly incidence of 0.7%.

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Ott et al., 2000), ctd.	40 records were not found (16 of the study group and 24 of the reference group)		1969-1973: < 10 ppb in most areas with 60 to 80 ppb in certain areas 1976-1988 (personal 8 h samples, paper type method): 5.9 ppb (average) 1989-1997 (personal 8 h samples, filter method); 2.8 ppb (average) JEM: Industrial hygiene measurements were linked to job-specific work history per person; peak exposure and 8 h TWA concentration were aggregated on a job- and time-specific basis for three job groups (potentially low/moderate/high TDI exposure); cumulative dose estimates (ppb-months) Average TDI concentration: < 5 ppb for 59% of the workers Cumulative TDI dose: < 500 ppb-months for 89% of the workers Frequencies of peak exposure > 20 ppb per shift: 0.5 in moderate exposure jobs, 0.9 in high-exposure jobs	Cumulative incidence for people assigned to TDI unit for at least 20 yrs: 11.5% (95% CI 5.3-17.7%) 7 of 19 cases had reported previous incidents of exposure to TDI (two related to rashes that had developed while handling TDI or waste products containing TDI) **Respiratory symptoms**: Since 1980 a standardised questionnaire was used that contained four questions with dichotomous answers (concerning wheezing/cough/chest discomfort/shortness of breath). No significant associations with responses in the questionnaires were found for those exposed to TDI versus referents. **Lung function** (spirometry): Neither cross-sectional nor longitudinal analyses of FVC and FEV_1 showed significant dose-response findings relative to exposure to TDI across the total exposed population.	Peak exposure and dermal exposure make it difficult to evaluate the 8h-TWA. Smoking, non-occupational asthma and allergy were assessed. Exposure to phosgene

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Bodner et al., 2001)	Longitudinal, data taken from routine medical surveillance examinations offered every 1 to 2 years Cross-sectional analyses (symptoms before entry and at last examination) Data from 1971-1997, mean follow-up ca. 8 years Dow Chemical, Texas, USA 305 TDI-exposed workers 581controls (hydrocarbons department)	TDI Manufacture	Mean observation period of TDI workers 7.8 years (SD 6.2) 449 8 h TWA TDI samples in 20 job categories; mean TDI exposure values per category calculated for start-up period (1971-1979) and full production period (1980-1997); individual work histories were matched to the 20 job categories to produce average exposure estimates and cumulative exposure estimates for each work segment for each worker Mean TDI concentration per individual: 2.3 ppb (SD 1.0), max. 5.2 ppb Average cumulative TDI exposure: 96.9 ppb-months (SD 110.6), max. 639 ppb-months Quartiles of the cumulative TDI estimates: 1-29 ppb-months, 30-70 ppb-months, 71-133 ppb-months, > 133 ppb-months Exposure categories with cut-points at 1 ppb for 1, 5, and 10 years, expressed in ppb-months (distribution for all observations): 1-12 (8.3%), 13-60 (36.6%), 61-120 (27.1%), > 120 (27.0%)	**Clinical symptoms** (questionnaire):One of the symptoms significantly more prevalent in controls than in exposed subjects at baseline (shortness of breath). Prevalence for all symptoms increased in both groups over time. Prevalence of symptoms not higher in TDI exposed subjects compared to controls at final examination. No effect of TDI on clinical symptoms reported during the study period found in regression models using four cumulative exposure categories or using a continuous cumulative variable or using quartiles of exposure. **Lung function** (spirometry):Average annual decline in FEV_1 was 30 mL. No association of TDI and decline in lung function found with mixed regression models using different exposure terms and subgroups.	Longest follow-up time (together with Ott et al. 2000) for TDI workers until then. Retrospective (change of formats of health surveys) Not enough exposure samples to derive annual TDI concentration estimates for each year for each job category Regression analyses for symptoms were adjusted for observation period and pack-years. Covariates considered for the mixed models for longitudinal lung function change were initial FEV_1, initial FVC, age, observation period, height, race, sex, race, entry period, pack-years, asthma, shortness of breath No exposure to MDI (as in some foam-manufacturing operations)

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Clark et al., 2003)	17-year longitudinal 1981-1998 UK Follow-up of Clark et al. 1998 7/12 factories remained n = 251 (217 were in the previous study)	TDI Manufacture of PU foam	Personal measurements: n = 1004 valid 1.3% in excess of 46.4 ppbh (5.8 ppb, 0.02 mg NCO/m^3) Respiratory protection taken into account by subtracting 50% of calculated exposure values Average daily dose for each exposed job at each factory calculated from the current and previous measurements Mean exposure for the period: Exposed group (n = 175): 8.4 ppbh Handling group (n = 26): 4.8 ppbh Low exposure group (n = 11): 2.3 ppbh	Longitudinal decline in **lung function** (same spirometer as in previous study; earliest measurement during 1981-1986 + further measurement in 1997/1998 used): Significantly higher loss in FEV_1 and FVC in handling group vs. low exposure group. Annual decline of FEV_1 and FVC not associated to TDI exposure. **Respiratory symptoms** (questionnaire): Differences in prevalence of respiratory symptoms between initial and final survey (reduction in some, increase in other symptoms).	Study was not designed to identify cases of sensitisation Persons showing evidence of TDI sensitisation would be removed and would no longer be available for study High attrition rate Respiratory illness was the reason for leaving in 2.3% of cases 70 subjects out of 251 (28%) changed groups during the 17-year period Number of present smokers fell from 129 (51%) to 100 (40%) between the two studies Only two data points used for lung function decline
(Dragos et al., 2009)	Prospective inception cohort study, 18 months n = 385 apprentice car-painters recruited between 1999 and 2002, complete data for n = 298	HDI monomers (and oligomers)	Personal breathing zone samples (n = 51) during regular and specific activities Area sampling (n = 41) in spray cabins and workplace background Duration for effective exposure to HDI max. 7 months, median 3 months	Health assessment included: - Respiratory symptoms (questionnaire) - Lung function (spirometry) - Metacholine challenge - Skin prick tests (only first visit) - HDI-specific IgE, IgG and IgG4	Subjects lost to follow-up 21.5% Short observation period Pre-exposure possible No individual exposure estimates

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Dragos et al., 2009), ctd.	First visit upon entry and second visit at the end of the training programme Montreal area, Canada		Median (maximum) concentration in µg/m^3, personal samples: Monomer: Spraying 0.001 (0.006) Mixing 0.0003 (0.0003) Brush cleaning < LOD (Oligomer: Spraying 0.283 (0.916) Mixing 0.4365 (0.6890) Brush cleaning 0.079 (0.079)) Concentrations from area sampling were lower than from personal sampling	Aims: - describe changes in specific antibodies to HDI - describe incidence of work-related symptoms - examine association between work-related symptoms and changes in specific antibody levels, and other potential risk factors Increases in specific IgE and IgG levels > 97th and 95th percentile were significantly associated with duration of exposure (nine subjects increased their IgG levels /IgE levels above the cut-off of the 97th percentile). Increases in specific IgG and IgG4 showed a protective effect on the incidence of work-related lower and upper respiratory symptoms, respectively. 13 subjects (4.4%) developed work-related respiratory symptoms, 19 (6.4%) developed work-related symptoms of rhinoconjunctivitis. No association between change in IgE levels and incidence of symptoms.	Masks worn when spraying, but not always those recommended and often removed inappropriately for inspecting the work. In regression analysis (dependent variable: IgE or IgG) only duration of exposure was used, but no concentration. At the exposure level in this study and after a few months, a small proportion shows increases in HDI-specific IgG and IgE
(Cassidy et al., 2010)	Matched retrospective cohort study Expands on Hathaway et al. 1999 (includes an additional plant)	HDI Two plants manufacturing or producing monomer (and/or polyisocyan-ates)	Industrial hygiene personal samples If record indicated that respiratory protection was used, sampling record was not considered	**Asthma** (annual medical surveillance history forms; suspect cases were inspected further by a company physician): No new asthma cases were reported.	No quantitative exposure estimations on the individual level Small number of exposure samples to reflect whole study period

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Cassidy et al., 2010), ctd.	Observation period: Plant 1 1988-2007 Plant 2 1987-2006 Southern US 57 potentially exposed in plant 1 and 43 in plant 2 (mainly exposed to HDI monomer) Controls: Plant workers without documented history of exposure to diisocyanates 1:1 matching by age, gender, race, smoking status, date of birth, date of hire		Mean (range): Plant 1, 237 samples 0.79 ppb (Non detectable – 31 ppb) Plant 2, 29 samples 0.3 ppb (Non detectable – 2 ppb) Most of the study group reported some instances of dermal exposure	Changes in **lung function** over time (annual spirometry), examined by a random coefficient regression model: Decline in lung function (FEV_1, FVC) over time in the exposed group was significantly greater than in the control group.	Smoking was assessed as binary variable. Controls may have been heavier smokers (significant difference in lung function decline between smoking controls and smoking exposed) Potential co-exposures reported: Exposed group: Other aliphatic diisocyanates, HDI polyisocyanates Control group from plant 1: dinitrotoluene, hydrazine, methylene chloride, maleic anhydride, toluene diamine, ethylene oxide Control group from plant 2: cerium, neodymium oxides, nitric acid, ammonia, kerosene, tributyl phosphate (depending on work area) No employee had to be medically removed because of HDI exposure Individuals with asthma were excluded from work with potential exposure (only in plant 1) and there may have been self-deselection.

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Gui et al., 2014)	Inception cohort study Evaluation of 49 newly hired workers pre-employment, after six and after twelve months Grouping of workers in exposure risk groups, based on potential risk of TDI exposure: low n = 8, medium n = 28, high n = 13.	TDI-based state-of-the-art PU foam production in Eastern Europe	Continuous fixed-point air sampling in foaming hall and cutting areas. 90% of the samples < LOD (0.1 ppb) Maximum recorded 10.0 ppb (foaming hall), 5.4 ppb (cutting area) No air sampling period exceeded an 8 h TWA of 5 ppb Peak exposures recorded were below 20 ppb. Personal sampling performed on seven workers. All showed TDI levels < LOD. Dermal exposure occurred (uncured or just cured foam, contaminated surfaces).	Over the first year of employment, 7 workers (14%) had findings that could indicate TDI-related health effects (Either new asthma symptoms, TDI-specific IgG, new airflow obstruction or a decline in $FEV_1 \geq 15\%$). Twelve workers (25%) were lost to follow-up. Among these workers, current asthma symptoms were reported (at baseline or 6 months) in a significant higher percentage compared to those who completed the 12-month follow-up. No significant associations were found between the exposure risk group and health outcomes. Self-reported glove use differed significantly between the exposure risk groups (25% of the workers in the low, 32% in the medium, 100% in high exposure risk group). Although this production facility is reported to be state-of-the-art with exposure below the OEL, the study suggests possible TDI-related health-effects.	Actual exposure of individuals is not known: TDI air levels may have been higher near the source. Dermal exposure occurred. Glove use differed between exposure risk groups. No unexposed control group No exposure quantification per exposed group Workers with spirometry data at baseline n = 23, with spirometry data at all three time points n = 16. Baseline spirometry conducted at another facility.

1.1.1.3 Case-control studies

The available case-control studies are summarised in Table 4.

Table 4: Case-control studies on respiratory sensitisation related to HDI, MDI, or TDI

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Tarlo et al., 1997)	Comparison of the level of isocyanate Concentration in 20 “case companies” (with compensated isocyanate asthma claims) with 203 “non-case companies”	HDI, MDI, TDI (or more than one)	Exposure data taken from a database of the Ontario Ministry of Labour (MOL): Air samples collected during the same 4-yr period during which the OA claims arose. Exposure determined on the basis of the highest level identified. Two categories: Always < 0.005 ppm Ever ≥ 0.005 ppm	56 accepted claims for OA (OA cases with identified isocyanate exposure during the 4-year period from mid-1984 to mid-1988 in the Ontario Workers’ Compensation Board) Combined across isocyanate types: Companies with claims in the high exposure category: 10/20 (50%) Companies without claims in the high exposure category: 50/203 (25%) OR = 3.1 (95% CI: 1.1–8.5, p = 0.03). MDI: OR = 1.7 (95% CI: 0.4–7.6) TDI: OR = 2.7 (95% CI: 0.7–10.6) Estimated incidence of OA in a 4-yr study period: High exposure companies with claims: 2.7% Low exposure companies with claims: 2.2% Overall incidence in the total 223 companies surveyed: 0.9% (56 out of 6308 workers).	Many high exposure companies without claims. Other factors may be important in isocyanate sensitisation, or there may have been quantitative or qualitative differences in exposure that were not assessed. Selection bias possible (some of the air sampling conducted in investigation of submitted claims for OA) Companies with claims had more employees than those without claims (higher probability of at least one employee becoming sensitized in a greater group of employees; larger companies may be more likely to implement a surveillance program).
(Meredith et al., 2000)	Company A: 27 OA cases were matched to 51 references (sex, work area)	Company A: 24 cases attributed to TDI (manufac-ture of moulded and block flexible PU foam, flame bonding and surface coating of fabrics);	Company A: Personal exposure measurements by job category (1979-1986) made for a separate study + data collected after 1986 by occupational hygiene consultants were used to estimate 8h-TWA and	**Asthma** Data from the two sites were analysed separately. Company A: Conditional logistic regression: 8 h TWA as a binary variable (cut off: median concentration in control group) or continuous variable 0.1 ppb increments)	Uncertainties in exposure assessment Regression analyses adjusted for smoking and different atopic diseases

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Meredith et al., 2000), ctd.	Company B: 7 cases; all non-cases (n = 12) served as controls, because matching was not possible (moving between work areas, few workers)	3 cases attributed to MDI (batch moulding of rigid PU components at 200°C) Company B: Cases attributed to MDI from a chemical plant in which MDI and poly-merric MDI mixtures were pro-cessed and poured into drums. Some processes involved heating the mixtures.	peak exposure for each subject based on job title and date. Company B: Personal monitoring results from 1988 available (Marcali method to the middle of 1990, HPLC thereafter) For each subject, the proportion of measurements ≥ LOD of the Marcali method (2 ppb) and > 5 ppb were calculated. Measurements < 2 ppb were treated as being 0. 90% of the 269 TWA samples were < 2 ppb	Peak exposures: 1 – 50 ppb In 31 subjects peak exposure > 20 pbb No difference between cases and controls. Mean 8-h TWA: cases: 1.5 ppb; controls: 1.2 ppb OR for exposure > median of the control group: 3.2 (95% CI 0.96 – 10.6; p = 0.06) Adjusted OR (for 0.1 ppb increase in 8h-TWA): 1.07 (95% CI 0.99 – 1.16) Adjusted OR higher for smoking (2.4) as well as history of either hay fever, eczema or asthma (3.4), but also n.s. Company B: Association between reported chemical accidents and asthma.169/185 TWA samples for controls and 74/84 for cases were < 2ppb. Mean and median exposures were < LOD for cases and controls. Median of the highest concentration recorded for each subject was 3 ppb for both groups. Proportion of measurements ≥ 2 ppb was 0.09 (controls) and 0.18 (cases). Proportion of measurements > 5 ppb was 0.004 (controls) and 0.09 (cases). 3/7 cases and 1/11 controls had at least one 8h-TWA exposure measurement > 5 ppb (OR 7.5; p= 0.09)	Amines are used as catalysts in the manufacture of PU foams and they have been reported to cause respiratory symptoms

1.1.1.4 Cross-sectional studies

The available cross-sectional studies are summarised in Table 5 and Table 6.

Table 5: Cross-sectional studies with quantitative exposure-response estimates on respiratory sensitisation related to HDI, MDI, and/or TDI

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Pronk et al., 2007)	n = 581 (241 spray painters, 50 unexposed office workers, and 290 others) Workplace survey in several companies between 2003 and 2006	HDI monomer and trimers in spray-painting (car body repair shops, furniture paint shops, industrial paint shops specialising in ships and harbour equipment or airplanes)	Personal exposure estimates were obtained combining personal task-based inhalation measurements for 23 different isocyanate compounds and time activity information Exposure of 241 spray painters, [µg NCO * m-3 * h * mo-1], median (min-max): Total isocyanate 3,682 (4-66464) HDI 27 (0.2-1427) (Biuret 269 (0.2-13568) Isocyanurate 2250 (6-87623))	Prevalence ratios (PR) and 95% CI for an interquartile range increase in exposure were calculated based on log-transformed exposure data. **Respiratory symptoms** (grouped into “asthma-like symptoms” and “COPD-like symptoms”), work-related symptoms (questionnaire): Respiratory symptoms were more prevalent in exposed workers than in office workers. Significant positive log-linear exposure-response associations were found for: Asthma-like symptoms PR (95% CI) = 1.2 (1.0-1.5), COPD-like symptoms 1.3 (1.0-1.7), Work-related chest tightness 2.0 (1.0-3.9) and Work-related conjunctivitis 1.5 (1.0-2.1), but not for Work-related rhinitis 1.3 (0.9-1.7) Different HDI-specific (for monomer and oligomers) IgE and IgG antibodies:	For subsample with BHR see (Pronk et al., 2009) Prevalence ratios were adjusted for age, sex, current smoking and atopy (or some of those) Possible effect modification by atopy was explored

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Pronk et al., 2007), ctd.				Prevalence of specific IgE antibodies was low (up to 4.2% in spray painters). Prevalence of specific IgG was higher (2-50.4%). One of five specific IgE antibodies and four of five specific IgG antibodies were positively associated with exposure. **Bronchial hyperresponsiveness (BHR)** assessed by methacholine challenge in a subset of 229 workers. Individuals with asthma-like symptoms were more likely to have BHR: PR (95% CI) = 2.2 (1.5-3.2). For COPD-like symptoms, the association with BHR was less strong and n. s.	
(Pronk et al., 2009)	Subset of study by Pronk et al. 2007 229 workers from 38 companies (91 spray-painters, 20 unexposed office workers, 118 others)	HDI monomer (and trimers) in spray-painting	Personal exposure estimates were obtained combining personal task-based inhalation measurements for 23 different isocyanate compounds and time activity information Exposure of 91 spray-painters, [µg NCO/m^3 x h/mo], median (min-max): Total isocyanate 4530 (15.4-66464) HDI 36.2 (1.3-472)	Prevalence ratios (PR) and 95% CI for an interquartile range increase in exposure were calculated based on log-transformed exposure data. **Lung function**: Highly exposed workers had lower FEV1, FEV1/FVC and flow-volume parameters. Percentage of workers who met the Global Initiative for Chronic Obstructive Lung Disease (GOLD) criteria for COPD (FEV1/FVC <70%): Office workers 5, other workers 4, spray-painters 15. COPD clearly associated with exposure. PR (95% CI): 2.7 (1.1-6.8) **Bronchial hyperresponsiveness** (BHR) (defined as a provocative cumulative dose of methacholine of ≤ 2.5 mg (~10 µM) required to cause a 20% fall FEV1): Percentage of workers with hyperresponsiveness (BHR20): office workers 0, other workers 14.7, spray-painters 20.	Associations were adjusted for age, sex, current smoking and atopy Associations for lung function parameters: additionally adjusted for height and race Strengths: Quantitative inhalation exposure assessment based on > 500 measurements and detailed task activity information; Several objective respiratory effect measures investigated in one population Limitations: Use of personal protective equipment, previous exposures and dermal exposure was not taken into account; Complex exposure environment; Healthy worker effect possible

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Pronk et al., 2009), ctd.				Hyperresponsiveness was found in 33 subjects and it was clearly associated with exposure expressed as total NCO. PR (95% CI): 2.0 (1.1-3.8) (adjusted for smoking, age, sex and atopy) BHR combined with asthma-like symptoms was present in 19 subjects and the adjusted PR was 2.7 (1.0-6.8). **Symptoms** (see (Pronk et al., 2007)): Asthma-like symptoms, COPD-like symptoms, work-related chest tightness were more prevalent among workers with higher exposure (n. s.). Workers with asthma-like symptoms had sign. more BHR, sign. lower baseline FEV1, FEV1/FVC and maximal mid-expiratory flow. No sign. association between exposure and exhaled nitric oxide (eNO) IgE and IgE (see (Pronk et al., 2007)): The prevalence of specific IgE antibodies was low (< ~4.4%). The prevalence of specific IgG was higher (up to 47% in spray painters). Specific IgG sensitisation was more common in highly exposed workers. Workers with specific IgE/IgG were more often hyperresponsive (overall; statistically significant only for one IgG). *"The current study provides evidence that exposure to isocyanate oligomers is related to asthma with bronchial hyperresponsiveness as a hallmark, but also shows independent chronic obstructive respiratory effects resulting from isocyanate exposure."*	

Table 6: Further studies - cross-sectional studies

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Bruckner et al., 1968)	Cross-sectional n = 26 with multiple exposures to diisocyanates n = 18 had never worked with or around isocyanates	TDI, polymeric isocyanates including MDI, xylylene diisocyanate Research, development and production of isocyanates and other components of urethane plastics	Exposed workers had accumulated exposure from 3 months to 11 years Air samples taken by industrial hygienist, modified Marcali method. Between 3 and 79 samples per year for single years between 1957 and 1967. Median concentration per year: 0-77 ppb	**Symptoms** (interview, physical examination) Immunologic reactivity to isocyanate antigen conjugates (several tests) Four groups: - Exposed minimal response (minimal symptoms of mucous membrane irritation) n = 5 - Exposed overdose response (moderate to marked signs and symptoms of chemical irritation of the respiratory tract) n = 16 - Exposed sensitised (signs and symptoms of sensitisation) n = 5: With increasing number of exposure, the time to reaction became shorter and finally bronchospastic symptoms developed within seconds after exposure to minute amounts of isocyanates. All had irritative symptoms before developing symptoms indicative for sensitisation. All had exposures > 20 ppb. - Non-exposed n = 18 6 cases of irritant dermatitis Workers exposed to low levels (not given) of isocyanates developed eye, mouth and throat symptoms. According to the authors concentrations between 20-100 ppb “may predispose some workers to sensitivity to isocyanate compounds”	Groups built based on exposure and type of response
(Wegman et al., 1974)	Cross-sectional 1972 Before and after shift on a Monday after three days away from work n = 111 (78 males)	TDI Manufacture of PU for matresses and auto seat cushions	Area sampling on the day of lung function testing and on three subsequent days (Marcali method, (Marcali, 1957)) All job areas were sampled and assigned exposure values and each worker was categorised according to his or her exposure to a measured mean concentration of TDI.	**Lung function** (spirometry: FEV_1, FVC; in the morning before work and in the afternoon after eight hours work; only FEV_1 reported): All exposure groups showed significant loss in lung function (FEV_1) during the working day. Dose-response relationship suggested (mean change in FEV_1 0.078 L in group A and 0.180 L in group D). Confirmed by regression analyses. And con-firmed by calculation of ratios of those showing no change or increase over those showing decrease per exposure group (ratio increases with exposure group).	Followed up: (Wegman et al., 1982; Wegman et al., 1977) Age, height, years smoked, cigarettes smoked, duration of exposure was considered for stepwise regression analysis

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Wegman et al., 1974), ctd.			Originally exposure categories were combined to four groups (ppm): A 0.002 - 0.003, B 0.004, C 0.005, D 0.006 – 0.013	Greater fall in FEV_1 in workers with symptoms compared to workers without symptoms, n. s. No trend of FEV_1 across subgroups of age, years of smoking or years of employment.	
(Pham et al., 1978)	Cross-sectional Two factories producing mainly plastic foam automobile accessories 318 workers (214 men) who had been employed for at least a year	MDI PU foam moulding	Workers used MDI and some TDI for 1 to 10 years. Plant A: MDI consistently < 20 ppb Plant B: MDI peaks up to 87 ppb at foam injection workplaces Group I: Not exposed to any occupational hazard n = 83 (62 men) Group II: Indirect exposure risk due to foam plastics manufacture n = 117 (61 men) Group III: Definite, direct exposure risk due to foam plastics manufacture n = 118 (91 men)	**Lung function** (single breath carbon monoxide transfer factor test, spirometry): Lower values of VC and diffusion constant in the exposed groups and associated with length of exposure. Possibility of fibrosis in workers with long exposure suggested. Results for men not confirmed by results for women. **Respiratory symptoms** (questionnaire): Higher frequency of bronchitis in exposed groups compared to unexposed group (men and women).	Followed up by (Pham et al., 1988) Exposure on factory level Men and women analysed separately Exposure to stripping agents, solvents, polyvinyl vapour in exposed groups Exposure to TDI No statistically significant differences between the groups concerning age, height, weight, smoking. More men smoke than women and they are heavier smokers.
(Holness et al., 1984)	Cross-sectional, shift, intraday, intraweek 1982 Toronto area Four companies	TDI Use in foaming operations	Mean length of exposure to isocyanates of 6.5 years Monitoring of TDI and respirable dust during same shift as lung function analysis (area samples; personal samples for 86 workers)	**Lung function** (spirometer, beginning and end of work shifts on Monday, Wednesday, Friday, sitting position using noseclips): Values of all lung function parameters (Monday morning) lower in the exposed than in the control group (not significant, adjusted for smoking). Significantly larger declines in lung function over the shift in exposed workers.	Respirable dust, mean for all exposed: 0.30 mg/m^3 Significantly lower frequency of family history of asthma, hay fever, bronchitis in exposed group (may be due to screening prior to employment or workers with positive family history may have developed symptoms and left).

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Holness et al., 1984), ctd.	95 isocyanate-exposed workers (70% males, 26 foam-line, 11 injection, 28 finishing, 21 miscellaneous) 37 control workers (62% males; 16 plant, 21 Ministry of Labour) (29 were excluded)		Mean exposure concentration for five groups of workers: Area: 0.1 – 1.8 ppb Personal: 0.6 – 2.1 ppb Mean for all exposed: Area: 0.6 ppb Personal: 1.2 ppb Some analyses with three exposure categories: control, ≤1ppb, >1ppb One personal sample > 20 ppb Less than 3% of the personal or area values > 5 ppb	Decline in FVC and FEV_1 over the shift increased over the three exposure categories, but was statistically significant only between controls and exposed groups. No significant relationships observed in regression analysis with continuous exposure. **Respiratory and further symptoms**: Slightly higher frequency of respiratory symptoms in exposed group, n. s..	
(Alexandersson et al., 1985)	Cross-sectional n = 67 (57 males) n = 56 controls (11 with lung function tests)	TDI, MDI Seven PU foam manufacturing factories (two foam PU blocks, five cast PU in moulds)	Personal sampling on same day as lung function tests Day mean exposure to TDI in foaming of PU blocks: for the whole group: 0.008 mg/m^3 (0.001 ppm) Highest exposure in the group working by foaming machine: 0.023 mg/m^3 (0.008-0.060) Day mean exposure to MDI ≤ 0.001 mg/m^3 during casting in moulds. Highest measurement: TDI 0.275 mg/m^3 MDI 0.139 mg/m^3	**Lung function** (spirometry: FEV_1, FVC, FEV%, MMF; nitrogen washout: Phase III, Closing volume; in the morning prior to work; exposed workers were studied again in the afternoon after work): Lung function of non-exposed group similar to reference values. Lung function of exposed group significantly impaired as compared to reference values, but significant in subgroup of smokers only. No significant changes during work shift. **Symptoms** (standardised questionnaire): Frequency of symptoms significantly higher in exposed non-smokers than in non-exposed non-smokers (nose, throat, dyspnea). No significant difference in symptoms frequency between exposed and non –exposed smokers.	To calculate day exposure figures < detection limit (0.001 mg/m^3) were set to zero. Selection bias (underestimation of acute adverse effects of TDI as sensible individuals may tend to terminate their employment)

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Venables et al., 1985)	Cross-sectional (Outbreak of asthma was investigated) 1979 n = 221	TDI Steel coating plant; continuous process, coat was cured by passage through an oven	TDI: 14 ppb at oven entry during normal processing, up to 26 ppb during 5 minute stoppage TWA 1979: 20 ppb	21 workers (9.5%) with OA symptoms (questionnaire) in 7 years (onset of symptoms after 1971) Symptomatic groups had significantly lower FEV_1 than asymptomatic group. TDI was found to be the cause of the asthma outbreak. It was liberated by a coating modified by a supplier in 1971.	No individual exposure levels Affected individuals may have left the plant
(Alexandersson et al., 1987)	Cross-sectional and over workweek 15 garages in Stockholm area n = 41 car painters n = 48 car platers (exposed to solvents, grinding dust, welding fumes like car painters, not to isocyanates n = 70 car mechanics Car painters and platers were matched against a control by sex (only males), age, height, and smoking	HDI Monomer (and biuret trimer) Car painters working with polyurethane paints	Exposure questionnaire Exposure monitoring 278 samples of HDI (and HDI-BT) Exposure has been individually related to time, use of respiratory protections, working operation, ventilation. Individual exposure determined by industrial hygienist HDI: 1.0 µg/m^3 (HDI-BT for car painting: mean (range): 115 µg/m^3 (10-385) High short-term peaks up to 13500 µg/m^3 HDI-BT)	Exposed workers were examined on Monday morning before work and on Friday afternoon Change in **lung function** within the week (spirometry: FEV_1, FVC, maximum mean expiratory flow MMF; Nitrogen washout: Phase III, Closing volume): Car painters did not differ from controls in any of the spirometric variables (before the workweek). Closing volume percent was significantly higher in exposed than in control workers. No significant difference in lung function in car painters before and after a workweek. **Symptoms** (interview by a nurse, standardised questionnaire): Eye, nose, throat irritation more frequent in car painters and platers than in controls, significant for platers only.	Uncertainties in exposure assessment Selection bias (some car painters had been relocated or their employment terminated)

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Wang et al., 1988)	Cross-sectional 1985 Taiwan n = 34, mostly females (38/45 workers had complete data, 4 were excluded because of smoking history) Follow-up (five months after recommendations for improvement of worker protection by the study team)	TDI Velcro-like tape manu-facture	Average length of employment 9.2 months Air samples, mean: Weaving (n = 3) 12 ppb Packaging/storage (n = 3) 21 ppb Tape processing (n = 15) 47 ppb Highest concentration measured: 236 ppb 5 months after improvement: 7 of 9 air samples < 7 ppb at the processing area	**Lung function** (spirometry in the morning, during a usual working day, after 10 days holiday,5 months after improvement of the workplace): Lung function of n = 21 workers after 10 days holiday: Greatest changes in pre- and post-exposure FEV1 and FVC for workers in the processing areas **Asthma** or asthmatic bronchitis (defined by development of cough for more than 1 month and shortness of breath or wheezing for 1 month after working in the factory):14 workers met the case definition of asthma or asthmatic bronchitis. Overall prevalence of asthma = 14/34 = 41.2% Significant trend in asthma frequency across the three exposure areas (0 cases in weaving, 37.5% in packaging/storage, 84.6% in tape processing). Follow up (5 months): No asthmatic symptoms. Lung function significantly improved (FEV_1 and FVC) for 10 workers still employed.	No unexposed control group Difficult to distinguish between irritant and allergic reactions Reversibility may be due to irritant effect and due to short exposure duration. High turnover rate
(Olsen et al., 1989)	Cross-sectional Dow, Texas, USA n = 57 manufacturing workers (85% participated) n = 89 unexposed workers (89% participated)	TDI Manufacture operations	Average TDI plant experience 4.1 years (< 1 – 9 years) Routine industrial hygiene measurements: TWA < 5 ppb, short-term exposure level 20 ppb for routine plant processes Use of self-contained breathing apparatus for breaking into lines for employees. Potential exposure was ranked by an industrial hygienist: None, low, moderate, high	**Lung function** (spirometer, after at least two days away from work, standing or sitting, without the use of nose clips): TDI exposure (classified as current, highest, cumulative, cumulative highest-to-date) not associated with decline in FEV_1 **Respiratory symptoms** (questionnaire): Prevalence of upper respiratory symptoms 68% in nonexposed group, 34% in exposed group Prevalence of lower symptoms 33% in nonexposed group, 17% in exposed group	No individual exposure levels Age, height, smoking considered in regression analysis Exposure misclassification possible, because rankings were applied to jobs regardless of calendar time

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Huang et al., 1991)	Cross-sectional 1988-1989 Asia 48 workers (25 males) in three factories: Factory A n = 15 Factory B n = 29 Factory C n = 13 18 controls (9 males)	TDI Furniture manufacture factories; painters exposed to TDI aerosol while brushing PU varnish to the surfaces of wood furniture	Area sampling at five spots Day mean exposure calculated from four measurements taken one, three, five, seven hours after the start of the work shift Marcali method Mean (range): Factory A: 0.79 mg/m^3 (0.49-1.18) Factory B: 0.31 mg/m^3 (0.22-0.89) Factory C: 0.11 mg/m^3 (0.07-0.24) Aerosol Dermal exposure likely (at least in factories A and B)	**Lung function** parameters (spirometry): Impairment of some lung function parameters significant in workers of factories A and B compared to the control group. **Symptoms** of the respiratory tract, skin, eyes (structured questionnaire administrated by occupational physicians): Prevalence of symptoms was significantly higher in factory A as well as in factory B compared to the control group. No significant difference was detected between workers in factory C compared to the control group. Symptoms of the eyes, nose, throat in all workers in factory A, 60% in factory B. No symptoms of the eyes in factory C and in the control group, 11 to 15% reported symptoms of the nose or throat. **Asthma-like symptoms** (dyspnea and wheezing during work):4 workers (26.7%) in factory A 3 workers in factory B (15%) no subject in factory C and of the control group. **Patch test** (0.1% TDI): Positive patch test in 5 and 2 painters in factories A and B (including three and two workers with contact dermatitis, respectively) and no subject in factory C or the control group. **Mast cell degranulation test**: Significantly higher mast cell degranulation percentage (MCDP) in painters from factories A and B than for the controls (specific to TDI-OA conjugates). No significantly higher MCDP in painters in factory C compared to the control group.	Cited in (Diller, 2002) Exposure measured only on one day and not on an individual level High exposure levels make it difficult to differentiate between irritant and allergic reactions. No information on potential differences in PSA between the factories. Medical history, smoking habits, duration of exposure, weight, height, age were assessed. No subject had a history of respiratory or skin diseases.

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Parker et al., 1991)	Cross-sectional Minnesota, USA 39 randomly selected autobody repair shops (out of 139 contacted shops 59 were eligible) 162 workers (160 males)	MDI, TDI Autobody repair	Mean number of years in autobody industry 11.4 ± 9.7 Isocyanate samples from 32 shops 8 h TWA total isocyanates: not detected to 60 ppb, mean 5 ppb Four percent of workers who spray-painted at least one hour/week never used a respirator, 33% sometimes, 63% always.	**Lung function** (spirometry at the start and the end of the work day): Abnormal lung function (< 5th percentile) in 8% (FEV1, FVC) and 23% (FEV1/FVC) of never smokers. No significant change in lung function between morning and afternoon shifts. Working-years in the autobody industry, nonfunctioning spray booth, smoking were associated with a decrement in FEV1/ FVC (regression analysis). No relationship between shop isocyanate concentration and lung function. **Respiratory symptoms** (self-administered questionnaire): Significant increase of wheezing across categories of respirator use (always, sometimes, never) while spray painting and for coughing and wheezing while sandblasting for non-smokers. No trends for respiratory symptoms and respirator use while sanding.	No individual exposure levels Exposure to dust, solvents
(Lee and Phoon, 1992)	Cross-sectional 26 exposed workers (“mixers”), 26 controls (workshop maintenance and field staff from government departments), matched by age, race, smoking state	TDI PU foam manufacture	24 personal breathing zone samples: Mean: 0.16 ppm Range: 0.01 – 0.50 ppm	**Lung function**: Mean diurnal variation in PEFR (in one week period): Significantly higher diurnal variation in PEFR in mixers than in controls. FEV_1/FVC significantly lower in exposed (83.0%) than in controls (89.3%)	Cited in (Diller, 2002) High exposure level Survivor population

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Lee and Phoon, 1992), ctd.				Mixers with ten or more years of exposure showed evidence of chronic airways obstruction. **Respiratory symptoms** (questionnaire): About 50% of mixers had eye irritation or cough during work (significant higher prevalence than in controls). No overt cases of OA	
(Omae et al., 1992)	Cross-sectional (4-year follow up see (Omae et al., 1992)) 1981 Japan 90 workers (male), 44 reference workers in the same factories	TDI PU foam manufacture	Working in PU foam factories for 0.5-25 years, mean 13.3 129 personal samples: Arithmetic mean: 3.2 ppb, geometric mean: 1.0 ppb , 90th percentile: 8.4 ppb, maximum: 26 ppb Short-term exposure peaks > 20 ppb in 16/129 samples	**Lung function**, change over working day (three methods: forced expiratory flow-volume test, respiratory impedance, airway resistance and specific airway conductance): No significant differences in lung function between PU foam workers and referents, except for lower PEF and%PEF in the exposed group. No change of lung function during work shift in both groups. **Symptoms** (questionnaire with interview): Significantly higher prevalence of respiratory symptoms, nasal symptoms, eye symptoms in the exposed workers.	Exposure to tertiary amines, organic tin compounds, polyols, silicon oil, dichloromethane, freons, flame-resisting agents, pigments etc. Possibly a survivor population Current smoking did not affect the results
(Bernstein et al., 1993)	Cross-sectional 1991 n = 243 (n = 175 males) 3-year old plant	MDI Urethane mould plant that had been designed to minimise exposure to MDI	Average duration of employment: 18.2 months (range: 0-32 months) Continuous monitoring of MDI area levels: < 5 ppb Occasional spills reported by workers, but not detected by monitors	Methods: Workers with at least one lower respiratory symptom (questionnaire) and workers with specific antibodies were instructed to perform serial PEFR studies for two weeks (n = 43). PEFR studies were also done in 23 control subjects (no symptoms, no antibodies). Workers with PEFR variability were evaluated by a physician (including methacholine test) for final diagnosis of OA/non-OA. Workers who were assigned final diagnosis of OA/non-OA/work-related urticaria were reevaluated in 1992 (n = 6).	No unexposed control group

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Bernstein et al., 1993), ctd.				Results: PEFR variability detected in 3/9 workers with questionnaire diagnosis of OA, in 2/4 workers with non-OA, in 2/23 control workers without symptoms. Three cases of physician-diagnosed OA (3/234, prevalence ca. 1%) and two cases of physician-diagnosed non-OA. Two workers had specific IgE and IgG to MDI-HSA. One of those had urticaria. Cases are considered to be due to intermittent higher than normal exposures to MDI during non-routine working activities. Cases were removed from exposure. After 1 year clinical status of OA was described as “inactive”.	
(Kim et al., 1997)	Cross-sectional Korea 81 workers (41 males)	TDI Spray painters Workshops manufacturing furniture or musical instruments or repairing motor vehicles	Area samples (n = 41) Range 0.5 – 10 ppb Mean 3.5 ± 2.3 ppb Four samples (9.8%) > 5 ppb	Examinations: Respiratory symptoms (questionnaires and interviews), Chest auscultation, IgE, IgG, FVC, FEV_1 Diagnosis of **TDI OA** was made if there was a decrease of PEFR over 20% of baseline and if the changing pattern was closely related to workshift. PEFR was recorded in the following cases: Subject complained of sputum, cough, and dyspnea aggravated by work, wheezing audible by auscultation, FVC or FEV_{10} < 80% of the normal Korean reference value, positive IgE RAST for TDI PEFR was checked for 15 workers. Eight workers (9.9%) were diagnosed with TDI-OA.	Cited in (Diller, 2002) No control group No individual exposure data

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Ulvestad et al., 1999)	Cross-sectional Norway? 19 injection workers (previous tunnel workers who were grouped into a department set up for sealing work; exposed to PU and acrylic resins; all the workers employed in this department in 1996 were included) 104 other tunnel workers, 6 different sites	MDI monomer (and prepolymer) Sealing work in tunnels	Job-years; mean (range): injection workers: 21 (1-42) tunnel workers: 13 (1-46) MDI monomer (personal sampling, 20 samples): mostly below the LOD (< 1 µg/m^3); 1.9 and 3.0 µg/m^3 at 2 occasions where isocyanate resin was spilled during injection work Pre-polymer: Four shift samples: 5.5 – 300 µg/m^3 (median 7.1); 18 short-term exposure values: 18-4300 (median 103) µg/m^3 Stationary sampling (n = 6): monomer < 4 µg/m^{3,} prepolymer < 4 - 31 µg/m^3	Examinations: Respiratory symptoms (questionnaire), lung function (spirometry), IgE (TDI, MDI, formaldehyde, eight common allergens), Metacholine provocation test, Clinical examination Higher prevalence of respiratory symptoms, airflow obstruction, BHR, asthma in injection workers compared to other tunnel workers. Two TDI-HSA-specific IgE positive injection workers (with work-related respiratory symptoms)	No exposure measurements available from the years the “injection department” had existed → most common exposure situations for workers during the last ten years were simulated. No individual exposure data Workers had not been informed about health hazards of the chemicals they worked with and did not report any use of airway protection. Exposure to acrylic resins Previous exposure to TDI Underestimation of exposure possible Years in the same job and smoking status were considered in the regression model
(Jang et al., 2000)	Cross-sectional Korea 64 randomly selected workers, 27 controls (23 males)	MDI (n = 20), TDI (n = 44) Petrochem-ical plant Manufacture	60 personal breathing zone samples Sampling during manufacture, sampling time 30-60 min Mean (maximum): TDI 17.4 µg/m^3 (42.9 µg/m^3) MDI µg/m^3 (6.4 µg/m^3)	Airway hyperresponsiveness (AHR) (definition: PC20 FEV_1 < 16 mg/mL of methacholine; continuous index of bronchial responsiveness: BRindex): Prevalence of AHR higher in MDI-exposed workers (4/20; 20%) than in TDI-exposed workers (2/42; 5%) and in controls (read from Figure: 2/27; 7%). Significantly higher BR index in MDI-exposed workers than in controls, but not significantly higher than in TDI-exposed workers. Differences statistically significant?	No individual exposure measurements Medication, work history, atopy, smoking was assessed by questionnaire

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Kakooei et al., 2006)	Cross-sectional Iran 39 employees in an automobile manufacturing company 117 unexposed employees at other work stations	MDI Window fixation, window glue processes	Personal samples Average concentration of MDI: Window fixation 34.53 µg/m^3 Window glue workplaces 27.37 µg/m^3	**Lung function**:%FEV1/FVC, %PEF significantly smaller in the exposed group than in the control group. **Respiratory symptoms** (questionnaire): Skin, respiratory, eye, mental symptoms significantly more prevalent in the exposed group. Respiratory, eye, mental symptoms significantly more prevalent in workers exposed to higher concentrations compared to lower concentrations than the mean value of 31.22 µg/m^3. Respiratory symptoms increased with the duration of service. However, symptoms not significantly correlated to years or intensity of exposure.	Occupational health and hygiene problems due to missing application of adequate engineering controls and proper safe work practice. Study was conducted in the summer. Higher exposure levels in the winter likely, because windows are kept closed then. No significant differences between the two groups in age, height, duration of service. However, duration of service was shorter in the exposed group. No information on smoking.
(Littorin et al., 2007)	Cross-sectional Southern Sweden n = 136 exposed to TDI in eleven plants n = 118 unexposed workers from different activities	TDI or TDI-based PU MDI used in 4/5 moulding plants (low or non-detectable). IPDI used in 1 of these plants.	Median personal 8 h exposure to TDI (ppb): continuous-foaming: 0.63-4.0 flame lamination: 0.76-1.5 molding: 0.17-0.64 low heating or nonheating processes: 0.02-0.05 Individual airborne exposure: measured during one shift (n = 79 workers), estimated based on department, task, air measurements (n = 57). Biomonitoring: 2,4-TDA and 2,6-TDA Urine: LOD – 623 and 353 noml/L Plasma: LOD-254 and 509 nmol/L	**Respiratory and eye symptoms** (structured interview, physical examination): Comparison between exposed and unexposed group: Total symptoms: significant increase in symptoms of the lower airways, nose bleeding (as the only nose symptom investigated), eye symptoms for the exposed group. Work-related symptoms: strong associations with exposure, in particular for attacks of eye symptoms (OR = 10), “wheezing etc” (OR = 21) and dry cough (OR = 11). Continuous measure of exposure within the exposed cohort: Only eye symptoms significantly associated with exposure measures (air, plasma, urine; OR from 1.6 to 4.2)	Symptoms may have been caused by combined exposures. Coexposures: dusts, other diisocyanates, organic solvents, thermal degradation products of ready-made PU in flame lamination plants (mix of mono-and diisocyanates, aminoiso-cyanates, amines) High number of workers with airway symptoms is seen as remarkable by authors, because of the selected work-force. However, no dose-res-ponse relationship with TDI. Individual airborne exposure was measured for a part of the workers only.

Reference	Study design and subjects	Isocyanate and use	Exposure	Results	Remarks
(Littorin et al., 2007), ctd.		5 moulding plants, 2 continous-foaming plants, 2 flame-lamination plants, 2 plants with low heating or non-heating processes	Correlations between air measurements and biomarkers in urine as well as biomarkers in plasma. Biomarkers in urine and plasma also correlated. Skin exposure certainly present	Effect of 2,4-TDI on the eyes was more pronounced compared to 2,6-TDI No clear patterns for other exposure-response relationships	Logistic regression model included age, gender, smoking. Atopy was considered. Preemployment health examinations should lead to a selected workforce in the Swedish PU industry (rather healthy concerning airway disease).
(Pourabedian et al., 2010)	Cross-sectional, shift Iran n = 43 car painters (healthy on enrolment) exclusion criteria: respiratory disorders including asthma, cigarette smoking, use of respiratory drugs	HDI Car body paint shop	Mean daily exposure: 15 minutes Mean daily HDI TWA air concentration in the breathing zone: 0.42 ± 0.1 mg/m^3 Mean weekly HDI TWA: 0.13 ± 0.059 mg/m^3	**Lung function**: Variation in PEF (peak flow meter, before and after the shift, over one week): Mean peak flow at the end of the shift on painting day was significantly lower than at the start of the shift 72% of the workers had >10% variation in PEF on painting days Effects of exposure remained till the day after painting Significant difference between the two days Significant correlation between HDI and percentage of decrease in peak flow as well as mean peak flow on painting day	High exposure levels No unexposed control group Questions concerning statistical analysis/ reporting of results Organic solutions

1.1.2 Animal data for the category source substances HDI, MDI, and TDI

Table 7 shows the complete list of animal studies initially considered for this dossier. Based on the test substance and route used for induction and further quality criteria (for details cf. main dossier), studies were selected for or excluded from further assessment.

Table 7: Overview (in chronological order) of available animal studies for diisocyanates and results of filtering for further assessment[1,2]

Species	Induction route	Induction agent	Effects observed	Elicitation route	Elicitation agent	Endpoint(s) assessed	Other reason for exclusion	Reference
GP RB RA	INH	TDIuc						(Niewenhuis et al., 1965)
GP	IDE							IUCL: (Bayer, 1968)
GP	TOP							IUCL: (Bayer, 1970)
GP	INH	HMDI						IUCL: (DuPont, 1971)
GP	INH	HMDI						IUCL: (DuPont, 1974)
GP	IDE							IUCL: (Duprat et al., 1976)
GP	IDE							IUCL: (DuPont, 1977)
GP	TOP	PIPDI						IUCL: (IBR, 1977)
MO	INH	2,4-TDI						(Sangha and Alarie, 1979)
GP	IDE+TOP	m-XDI						IUCL: (Huntingdon, 1980)
MO	TOP							(Tanaka, 1980)
GP	TOP							IUCL: (BRC, 1981)
GP	IDE TOP							(Karol et al., 1981)
MO	INH	HDI	Y	-		RF	One exposure < 1 d, no AB	(Sangha et al., 1981)
GP	IVE							(Bernstein et al., 1982)
GP	IPE SCU							(Chen and Bernstein, 1982)
GP	IDE IPE TOE TOP							(Karol and Magreni, 1982)
DO	ITR							(Patterson et al., 1982)
MO	INH	HDI-BT						(Weyel et al., 1982)
GP	IDE							IUCL: (Bayer, 1983)
GP	IDE+TOP							IUCL: (IBR, 1983a)
GP	IDE+TOP							IUCL: (IBR, 1983b)
GP	INH	TDI	Y	-		AB	-	(Karol, 1983)
				IDE	TDI	SS		
				INH	TDI-GPSA	RF		
GP	TOP							(Koschier et al., 1983)
GP	INA							(Tanaka et al., 1983)
GP	IDE							IUCL: (Bayer, 1984a)

[1] Studies deselected for further assessment are shaded grey, as are the fields explaining which criteria for inclusion based on test substance, route, or quality were not met (for details on the deselection strategy, cf. main dossier). If for a given induction agent and route a study contained experiments with negative test results as well as experiments demonstrating effects, only the latter have been further evaluated. Experiments with knock-out animals were not considered, since the aim of this review was to identify effects in healthy animals.

[2] For explanation of abbreviations cf. section 15 of the main dossier.

Species	Induction route	Induction agent	Effects observed	Elicitation route	Elicitation agent	Endpoint(s) assessed	Other reason for exclusion	Reference
GP	IDE							IUCL: (Bayer, 1984b)
GP	TOP							IUCL: (Bio-Dynamics, 1984)
GP	INH	m-TMXDI						IUCL: (Bio-Research Laboratories, 1984a; Bio-Research Laboratories, 1984b)
GP	IDE							(Chang and Karol, 1984)
GP	IDE+TOP							(Clemmensen, 1984)
RA	INH	2,4-TDI						IUCL: (Hazleton, 1984)
GP, MO	INH	HMDI						(Stadler and Karol, 1984)
MO	TOP+INH							(Stadler and Karol, 1984)
GP	IDE+TOP							IUCL: (Bayer, 1985)
GP, MO	TOP							(Stadler and Karol, 1985)
MO	TOP							(Tominaga et al., 1985)
MO	INH	HMDI						(Weyel and Schaffer, 1985)
MO	INH	MDI	Y		-	RF	One exposure < 1 d, no AB	(Weyel and Schaffer, 1985)
MO	TOP+ FCA							(Gad et al., 1986)
MO	INH	2,4-TDI						IUCL: (Hazleton, 1986)
GP	IDE							IUCL: (University of Louisville, 1987)
GP	INH	IPDI						IUCL: (University of Louisville, 1987)
MO	TOP							(Tanaka et al., 1987)
MO	TOP							(Thorne et al., 1987)
GP	INH	TDI	Y	INH	TDI-GPSA	AB, RF	-	(Botham et al., 1988)
GP	INH	TDIuc						(Cibulas et al., 1988)
GP	IDE							(Jin and Karol, 1988)
RA	INH	HDI	Y		-	IF	Only IF	IUCL: (Mobay, 1988)
RA	INH	TDI	Y		-	IF	Only IF	IUCL: (Union Carbide, 1988)/ (Tyl et al., 1999)
GP	INH	m-TMXDI	Y	INH	m-TMXDI-GPSA	AB, IF, RF	-	IUCL: (Union Carbide, 1988)
RA	INH	HDI	Y		-	IF	Only IF	IUCL: (Mobay, 1989)
MO	TOP	IPDI						(Stern et al., 1989)
RA	INH	TDI	Y		-	IF	Only IF	IUCL: (Union Carbide, 1989)/ (Tyl et al., 1999)
GP	INH	MDI	Y		-	AB	-	(Dearman and Botham, 1990)
				IPE	MDI-GPSA			
RA, MO	INH	m-TMXDI	Y		-	IF	Only IF	IUCL: (Union Carbide, 1990)
RA	INH	TDI	Y		-	IF	One exposure < 1 d, no AB	(Hesbert et al., 1991)
GP	INH	HDI trimer						(Pauluhn and Eben, 1991)
GP	IDE							(Pauluhn and Eben, 1991)
MO	TOP							(Dearman et al., 1992a)
MO	TOP							(Dearman et al., 1992b)
GP	INA							(Kalubi et al., 1992)
GP	IDE+TOP							IUCL: (Safepharm, 1992)
MO, RA	INH	m-TMXDI	Y		-	IF, RF	One exposure < 1 d, no AB	IUCL: (Union Carbide, 1992)
GP	IDE+TOP							IUCL: (Bayer, 1993)
GP	INH	TDIuc						(Huang et al., 1993)
GP	INH	TDI	Y	INH	TDI	IF	-	(Huang et al., 1993)

Species	Induction route	Induction agent	Effects observed	Elicitation route	Elicitation agent	Endpoint(s) assessed	Other reason for exclusion	Reference
GP	INH	TDI	Y	INH	TDI	AB, RF	-	(Aoyama et al., 1994)
GP	IDE							IUCL: (Bayer, 1994)
MO	TOP							(Hilton et al., 1994)
GP	IDE							(Pauluhn, 1994)
	INH	MDI	Y	INH	MDI	RF	-	
					MDI-GPSA			
		TDI			TDI			
					TDI-GPSA			
GP	IDE							(Rattray et al., 1994)
	TOP							
	INH	MDI	Y	INH	MDI	AB, RF, SS	-	
RA	INH	PMDI						(Reuzel et al., 1994a)
RA	INH	PMDI						(Reuzel et al., 1994b)
GP	IDE	HMDI						IUCL: (Bayer, 1995a)
GP	INH	MDI	Y	INH	MDI	AB, IF, RF	-	IUCL: (Bayer, 1995b)
GP	IDE							(Blaikie et al., 1995)
MO	TOP							(Hilton et al., 1995)
RA	INH	MDI	Y		-	IF, RF	-	IUCL: (Hoymann et al., 1995)
GP	INA	2,4-TDI						(Yamada et al., 1995)
GP	TOP							(Basketter and Gerberick, 1996)
GP	IDE							IUCL: (Bayer, 1996a)
GP	IDE							IUCL: (Bayer, 1996b)
	INH	PIPDI						
MO	TOP							(Dearman et al., 1996a)
MO	TOP							(Dearman et al., 1996b)
GP	INH	TDI	Y		-	IF, RF	-	(Gagnaire et al., 1996)
MO	TOP							(Karol and Kramarik, 1996)
GP	IDE							(Mapp et al., 1996)
GP	INA							(Niimi et al., 1996)
GP	IDE+TOP							IUCL: (NOTOX, 1996)
MO	INA							(Scheerens et al., 1996)
	TOP							
GP	INH	TDI	Y		-	IF	Only IF	(Ban et al., 1997)
GP	INH	TDI	Y		-	RF	-	(Gagnaire et al., 1997)
RA	INH	TDI	Y		-	IF, RF	One exposure < 1 d, no AB	(Huffman et al., 1997)
GP	IDE+TOP	m-XDI						IUCL: (Huntingdon, 1997)
GP	INH+IDE							(Pauluhn and Mohr, 1998)
	INH	TDI	Y	INH	TDI/TDI-GPSA	AB, IF, RF	-	
GP	IDE							IUCL: (Safepharm, 1998a)
GP	IDE+TOP							IUCL: (Safepharm, 1998b)
MO	TOP							(Woolhiser et al., 1998)
MO	INA							(Zheng et al., 1998)
GP	TOP							(Zissu et al., 1998)
RA	INH	PMDI						(Pauluhn et al., 1999)
MO	TOP							(Scheerens et al., 1999)
RA	INH	PMDI						(Pauluhn, 2000a)
RA	INH	HDI-IC						(Pauluhn, 2000b)
GP	IDE	PMDI						(Pauluhn et al., 2000)
	INH							
MO	TOP+SDS	2,4-TDI						(van Och et al., 2000)
MO	TOP	2,4-TDI						(Vandebriel et al., 2000)
GP	INA							(Ebino et al., 2001)
	INH	TDI	Y	TOP	TDI	SS	-	

Species	Induction route	Induction agent	Effects observed	Elicitation route	Elicitation agent	Endpoint(s) assessed	Other reason for exclusion	Reference
	ITR							
	TOP							
MO	SCU							(Matheson et al., 2001)
RA	INH	HDI-BT HDI-IC						(Pauluhn and Mohr, 2001)
RA	INA							(Zheng et al., 2001)
MO	TOP							(Haag et al., 2002)
RA	INH	PMDI						(Kilgour et al., 2002)
MO	INA							(Lee et al., 2002)
MO	SCU							(Matheson et al., 2002)
RA	INH	PMDI						(Pauluhn, 2002a)
RA	INH	HDI-IC PMDI						(Pauluhn, 2002b)
MO	TOP							IUCL: (Bayer, 2003a)
RA	INH	MDI	Y	-		RF	One exposure < 1 d, no AB	IUCL: (Bayer, 2003b)
MO	INA							(Lee et al., 2003)
GP	IDE+TOP							IUCL: (NOTOX, 2004)
MO	TOP							(Vanoirbeek et al., 2004)
RA	INH	2,4-TDI						(Kouadio et al., 2005)
MO	INH	TDI	Y	INH	TDImix	AB, IF, RF	-	(Matheson et al., 2005a; Matheson et al., 2005b)
GP	TOP							(Nabe et al., 2005)
RA	TOP							(Pauluhn, 2005)
RA	INH	PMDI						(Pauluhn et al., 2005)
	TOP							
MO	TOP							(Plitnick et al., 2005)
MO	INH	TDI	Y	INH ITR	TDImix	AB, IF	-	(Ban et al., 2006)
	SCU							
	TOP+ITR							
RA	TOP							(Pauluhn and Vohr, 2006)
	INH	PMDI						
MO	TOP							(Selgrade et al., 2006)
MO	TOP							(Farraj et al., 2007)
MO	TOP							(Lim et al., 2007)
RA	INH	HDI-IC PHDI/ PTDI						(Ma-Hock et al., 2007)
MO	SCU							(Sun et al., 2007)
MO	TOP							(Tarkowski et al., 2007)
MO	INH	HDI	Y	-		IF, SS	-	(Arts et al., 2008)
		IPDI						
		PIPDI						
		TDI	Y	-		IF, SS	-	
	TOP							
RA	INH	HMDI						IUCL: (Bayer, 2008a)
RA	INH	IPDI						IUCL: (Bayer, 2008b)
MO	ITR TOP							(Fukuyama et al., 2008)
RA	TOP							(Pauluhn, 2008a)
RA	TOP							(Pauluhn, 2008b)
RA	INH	IPDI trimer						IUCL: (BASF, 2009)
MO	INH	HDI	Y	-		IF, SS	-	(de Jong et al., 2009)
		IPDI						

Species	Induction route	Induction agent	Effects observed	Elicitation route	Elicitation agent	Endpoint(s) assessed	Other reason for exclusion	Reference
		TDI	Y	-		IF, SS	-	
	TOP							
RA	INA							(Svensson-Elfsmark et al., 2009)
MO	TOP							(Vanoirbeek et al., 2009)
MO	TOP							(Vanoirbeek et al., 2009)
RA	INH	NDI						IUCL: (Bayer, 2010)
MO	TOP							(Fukuyama et al., 2010)
MO	TOP							IUCL: (Bayer, 2011)
MO	INH	MDI TDI	Y	-		IF, RF	Only IF and sensory irritation	(Lindberg et al., 2011)
RA	INH	PMDI						(Pauluhn and Poole, 2011)
MO	INA							(Swierczynska-Machura et al., 2012)
MO	TOP							(de Vooght et al., 2013)
MO	TOP							(Song et al., 2013)
MO	TOP							(Woolhiser et al., 2013)
MO	TOP							(Nayak et al., 2014)
RA	INH	TDI	Y	-		RF	Only sensory irritation	(Pauluhn, 2014)
	TOP+INH							
MO	INA							(Swierczynska-Machura et al., 2014)
MO	TOP							(Liang et al., 2015)
RA	INH	HDI	Y	-		RF	Only sensory irritation	(Pauluhn, 2015)
		HDI/PHDI						
	TOP							
MO	TOP							(Pollaris et al., 2015)
MO	TOP							(Wisnewski et al., 2015)

In the following sections, one key study for each animal species is summarised in detail[3].

1.1.2.1 Pauluhn and Mohr, 1998

Study reference:

Pauluhn J. and Mohr U. (1998): Assessment of respiratory hypersensitivity in guinea pigs sensitized to toluene diisocyanate: A comparison of sensitization protocols. Inhalation Toxicology 10 (2), 131-154. DOI: 10.1080/089583798197790 (last accessed 2016-09-20)

Since the classification criteria for RS ask for inhalation (and not mixed intradermal and inhalation) exposure, only the experimental design and results for the two treatment groups with exclusive inhalation exposure are reported here.

Test type:

No test guideline was followed since none is available for this endpoint. Sensitisation in guinea pigs was induced by single inhalation exposure to TDI vapour with subsequent inhalation challenge with the homologous TDI–protein conjugate, immunoglobulin G_1 (IgG_1) antibody analysis, and histopathological examination of the lung. In order to distinguish specific from nonspecific respiratory response, guinea pigs

[3] Note: Text is a mixture of excerpts from the respective publications or IUCLID summaries and of text prepared by the DS. Direct use of original text is not specifically marked.

were subjected to additional acetylcholine (ACh) bronchoprovocation assays one day before and one day after the challenge with TDI.

Test substance:

Toluene diisocyanate (TDI, DESMODUR T80), an 80:20 mixture of the 2,4- and 2,6-isomers, source: Bayer AG, Leverkusen, Germany, EC number 247-722-4, CAS number 26471-62-5, degree of purity > 99.9% (identity of remaining < 0.1% not reported), batch number not reported.

Test animals:

Guinea pigs/Dunkin-Hartley/female, weight at study initiation: 250-350 g, age at study initiation not reported, 8 animals per treatment group, 16 animals in control group.

Administration/exposure:

Route of induction and challenge: inhalation; control group: pooled from a sham-exposed group (8 animals) and a group receiving intradermal injections of corn oil (vehicle control for additional experiments performed in this study, 8 animals); induction concentrations used in treatment groups: 136 or 220 mg TDI vapour/m^3 air; challenge 1: on day 20, unspecific challenge with acetylcholine (ACH); challenge 2: on day 21, specific challenge with 0.5 mg TDI/m^3 air for 30 min; challenge 3: on day 22, unspecific challenge with acetylcholine (ACh); challenge 4: on day 28, specific challenge with TDI-GPSA conjugate.

Results and discussion:

Following single 15 minute-inhalation nose-only exposure to TDI at two different dose levels, Dunkin-Hartley guinea pigs displayed an increased respiratory rate after specific challenge with TDI (day 21) and TDI-GPSA hapten-protein complex (around day 28). Four weeks into the test, production of TDI-specific IgG_1 antibodies was demonstrated in serum samples of exposed animals. On sacrifice one day after the conjugate challenge, increased influx of granulocytes in trachea, lung and lung-associated lymph nodes and an increased number of macrophages in lung tissue were demonstrated. The results are displayed in more detail in Table 8 below (Pauluhn and Mohr, 1998).

Table 8: Results indicative of respiratory sensitisation from (Pauluhn and Mohr, 1998)

Parameter		**Control**	**Group 1 (136 mg/m^3)**	**Group 2 (220 mg/m^3)**
Specific TDI challenge (day 21)				
Immediate onset respiratory hypersensitivity, duration of increase of respiratory rate [4]		19%	63%	63%
Immediate onset respiratory hypersensitivity, intensity of increase of respiratory rate [5]		25%	50%	38%
TDI-GPSA challenge (ca. day 28)				
Immediate onset respiratory hypersensitivity, duration of increase of respiratory rate [4]		6%	25%	38%
Immediate onset respiratory hypersensitivity, intensity of increase of respiratory rate [5]		6%	38%	38%
Serum antibody production (day 28)				
Highest serum dilution demonstrating positive TDI-specific IgG_1 antibodies		NA	1:100	1:100
Histopathology				
Trachea				
Influx of granulocytes	Moderate	19%	13%	38%
	Severe	0%	0%	50%**

[4] Fraction of animals for which the number of events with an increase in respiratory rate amounted to more than three times the standard deviation of the individual baseline (similar period during the pre-challenge phase), no significance testing reported.

[5] Fraction of animals for which the area under the (respiratory rate) curve exceeded three times the standard deviation of the individual baseline (similar period during the pre-challenge phase), no significance testing reported.

Parameter		Control	Group 1 (136 mg/m³)	Group 2 (220 mg/m³)
Influx of eosinophilic granulocytes	Moderate	19%	25%	38%
	Severe	0%	0%	50%**
Lung				
Increased number of macrophages		19%	63%*	75%
Influx of granulocytes (bronchi)	Moderate	0%	25%	38%*
	Severe	0%	0%	0%
Lung-associated lymph nodes				
Influx of granulocytes	Moderate	0%	13%	63%**
	Severe	0%	0%	0%

* $p < 0.05$; ** $p < 0.01$

1.1.2.2 Respiratory sensitisation in mice (Matheson et al., 2005a; Matheson et al., 2005b)

Study references:

Matheson J.M., Johnson V.J., Vallyathan V., and Luster M.I. (2005b): Exposure and immunological determinants in a murine model for toluene diisocyanate (TDI) asthma. Toxicological Sciences 84 (1), 88-98. DOI: 10.1093/toxsci/kfi050 (last accessed 2016-09-19); Matheson J.M., Johnson V.J., and Luster M.I. (2005a): Immune mediators in a murine model for occupational asthma: Studies with toluene diisocyanate. Toxicological Sciences 84 (1), 99-109. DOI: 10.1093/toxsci/kfi051 (last accessed 2016-09-20)

The results of this study have been published in two publications of which only the main study (Matheson et al., 2005b) is summarised below, as (Matheson et al., 2005a) primarily addressed mechanistic questions which are not of relevance for this CLH dossier. Text, tables and figures are reproduced from the original publications, with slight editorial modifications by the DS.

Test substance

TDI (80:20 molar mixture of 2,4:2,6 isomers provided by Bayer, USA, Pittsburgh, PA)

Test animals

Preliminary studies were conducted using several mouse strains including C57BL/6, BALB/c, and B6C3F1 mice. Since the C57BL/6 strain produced the most robust responses under the current exposure conditions, the strain was used in the current studies. Female wild-type C57BL/6 J and FcErIg knockout (B6.129-FcerIg5tmlRav.N12) mice, deficient in the g chain of the FcerI, FcgRI, and FcgRIII genes, were obtained from Jackson Laboratory (Bar Harbor, ME), and Taconic (Germantown, NY), respectively, at approximately 5 to 6 weeks of age. Upon arrival the mice were quarantined for 2 weeks and acclimated to a 12-h light/dark cycle. Animals were housed in microisolator cages in pathogen-free and environmentally controlled conditions at NIOSH facilities in compliance with AAALAC approved guidelines and an approved IACUC protocol (03-JM-M-005). Food and water were provided ad libitum.

Methods

Atmosphere generation

TDI vapours were generated by passing dried air through an impinger that contained 3 mL TDI. A computer-interfaced mass flow controller (Aalborg Instruments, Orangeburg, NY, model GFC-37, 0–20 LPM) regulated the TDI concentration in the chamber, while a similar mass flow controller (model GGC-47, 0–100LPM) regulated the diluent air. Temperature and relative humidity were monitored by a Vaisala transmitter (Vaisala Inc., Woburn MA, type HP-233) interfacing with the TDI and diluent air controllers in a National Instruments (Austin TX) data acquisition/control system. The generation system produced TDI vapour, free of TDI aerosol.

Real-time monitoring of the chamber atmosphere was performed using an Autostep™ continuous toxic gas analyzer (Bacharach, Inc, Pittsburgh, PA) with TDI concentrations never varying more than 10% in the study.

Induction regime

Mice were exposed to TDI by inhalation either of 20 ppb of TDI for 6 weeks, 5 days per week, 4 h per day (subchronic exposure), or of 500 ppb TDI for 2 h (acute exposure), in a 10 L inhalation chamber with only the heads of the animals extended into the chamber.

Challenge

Challenge (1 h, 20 ppb TDI) was performed on all groups 14 days following the last day of subchronic or acute exposure. The 6-week exposure period is the time during which sensitisation to TDI develops in the current models. Therefore, mice that were exposed to TDI during this 6-week period followed by challenged are, henceforth, referred to as ‘‘sensitised/challenged’’ groups.

Control groups

Three control groups were examined, including an air sensitised/air challenged, TDI sensitised/air challenged, and air sensitised/TDI challenged treatment group. As all control groups responded similarly, for convenience, only results from the air sensitised/TDI challenged control treatment are shown in the publication and are, henceforth, referred to as ‘‘controls’’ except in AHR studies, where values for all groups were reported.

Tissue collection

Groups of mice from each treatment group were sacrificed 48 h after airway challenge, using a CO_2 atmosphere, and lungs and nares were collected. Lungs were inflated with 10% neutral buffered formalin (NBF), and tissues were immersed in 10% NBF for 24 h, after which the nares were decalcified. The tissues were embedded in paraffin, serially sectioned, and stained with hematoxylin and eosin for histopathological assessment. PAS staining was performed to identify goblet metaplasia and Chromatrope 2R/Mayer’s Hematoxylin staining for eosinophil identification. The histopathological grading system was performed blinded and expressed on a 0–5 scale for each animal, with 0 representing no change, 1 = minimal, 2 = slight/mild, 3 = moderate, 4 = moderate/severe, and 5 = severe.

Additional groups of mice were sacrificed 24 h after challenge and utilised for bronchoalveolar lavage fluid (BALF) and blood collection. To obtain BALF, mice were anaesthetised with 50 mg/kg of pentobarbital, exsanguinated, and intubated with a 20-gauge cannula positioned at the tracheal bifurcation. Each mouse lung was lavaged three times with 1.0 mL of sterile HBSS and pooled. BALF recovery was 80 ± 5% for all animals. The BALF samples were centrifuged, and the supernatant frozen at -80 °C until enzyme analysis. The cells were resuspended at 105 cells/mL of HBSS, and 0.1 mL was used for cytospin preparations. The slides were fixed and stained with Diff-Quick (VWR, Pittsburgh, PA), and differential cell counts were obtained using light microscopic evaluation of 300 cells/slide. Total cell counts were performed with a haemocytometer. In replicate experiments, lungs were collected 24 h following challenge, and tissues were frozen in RNAlater (Qiagen, Valencia, CA) and stored at -80 °C for reverse transcription polymerase chain reaction (RT-PCR) analysis. Tissues frozen in liquid nitrogen were incubated with RNAlaterICE (Ambion, Austin, TX) at -20 °C for 24 h prior to RNA isolation.

Transfer experiments

Adoptive and passive transfer experiments were conducted to assess the role of specific immunity in the asthma response. For adoptive transfer experiments, single cell suspensions were prepared from groups of mice exposed to TDI for six weeks or air sham controls by gently pressing pooled lymph nodes (mediastinal and auricular) and spleens through a stainless steel screen. The cell suspensions were washed with HBSS(Gibco, Grand Island, New York), the cell number adjusted to 2 x 10^7 cells/mL, and aliquots layered onto Lympholyte-M (Accurate Chemical, Westbury, NY).

After centrifugation at 2500 rpm, the lymphocyte interface was collected and washed, and 5.0 x 10^7 cells in 0.5 mL volumes were injected intravenously into naive recipients. B or T cell depletion was conducted by incubating isolated lymphoid cells with either panT or panB Dynabeads (Dynal Biotech Inc., Lake Success, NY) at a 7:1 cell:bead ratio, according to the manufacturer’s instructions. The respective T and B cell populations were > 98% pure, as assessed by FACS analysis on a FACS Calibur (BD Biosciences, Palo Alto, CA) utilising anti-CD3 and anti-B220 FITC conjugated monoclonal antibodies (PharMingen, San Diego, CA). The resulting T and B lymphocyte populations were injected intravenously into naive recipients at a concentration of 2.9 x 10^7 cells and 2.5 x 10^7 cells, respectively, in 0.5 mL volumes. To measure TDI-specific serum activity, naive mice received an intradermal injection of 30 mL heat-inactivated (56 °C, 4 h) or non-heated pooled serum into the dorsum of the right ear from either TDI sensitised/challenged mice or control mice. Animals were challenged 24 h later with 1% TDI (in acetone:olive oil, 4:1) on the dorsum of the same ear, and the change in ear thickness was compared to the thickness pre-challenge. Additional groups of mice received an intravenous injection of 200 mL of either heated or unheated pooled sera from TDI sensitised/challenged or control mice. Twenty-four hours after intravenous lymphocyte or serum transfer, mice were challenged either by inhalation with 20 ppb TDI for 1 h or by a single application of 25 mL of 1% TDI (in acetone:olive oil, 4:1) onto the dorsum of the right ear, as previously described (Ebino, 1999). Respiratory responses including pathology (as outlined above) and airway responsiveness to methacholine (see below) were determined 48 and 24 h following challenge, respectively. The ear challenge response was determined by measuring the change in ear thickness from baseline pre-challenge ear thickness 24 h following TDI application. Cell proliferation in the draining lymph node was determined in an additional group of recipient mice using a modification of the local lymph node assay, as originally described by (Dearman and Kimber, 2000). Twenty-four hours after challenge, the mice were injected intravenously with 200 mL of ^{3}H-thymidine (specific activity 0.1 mCi/mL; Amersham, Piscataway, NJ), and incorporation of ^{3}H-thymidine into DNA in the draining auricular lymph nodes was measured.

Antibody detection

Total serum IgE was measured using a sandwich enzyme-linked immunosorbent assay (ELISA) as previously described (Satoh et al., 1995). Briefly, plates were coated with 5 mg/mL of rat monoclonal antimouse IgE (PharMingen). Serial two-fold dilutions of test sera, starting at a 1:5 dilution, were added and incubated with peroxidase-goat anti-mouse IgE (1:1000, Nordic Immunological Laboratories, Capistrano Beach, CA) and developed with ABTS substrate, 2,20-azinobis(3-ethylbenzthiazoline-6-sulfonic acid). Total serum IgE concentrations were derived from a standard curve obtained using murine monoclonal anti-DNP IgE (Sigma, St. Louis, MO). TDI-specific antibodies were detected by ELISA using a TDI-mouse serum albumin conjugate, kindly provided by Dr. Meryl Karol (University of Pittsburgh, Pittsburgh, PA), as previously described (Satoh et al., 1995). Serial two-fold dilutions of test sera, starting at a 1:5 dilution, were added to individual wells and incubated with peroxidase-conjugated, goat anti-mouse antibodies against either total IgG (1:400, Sigma, St. Louis, MO), IgG_1, or IgG_{2a} (both at 1:400, The Binding Site, Birmingham, UK) and developed with ABTS substrate. Antibody titers were determined by plotting the serial dilution curve for each sample individually vs. the optical density (OD) for each dilution of that sample. A cut-off OD of 0.2 (average OD of challenge only mouse serum was 0.06 ± 0.005) was used to determine the titer.

Eosinophil peroxidase activity (EPO)

Measurement of EPO activity was performed on BALF supernatants according to the method of (Bell et al., 1996), with slight modifications. Briefly, 0.1 mL of peroxidase substrate solution, consisting of o-phenylenediamine dihydrochloride (OPD), urea hydrogen peroxide, and phosphate-citrate buffer (Sigma Fast Tablets, Sigma, St. Louis, MO), was added to 0.1 mL of the BALF supernatant. The mixture was incubated at 37 °C for 30 min before stopping the reaction with 50 M of 2 N hydrochloric acid. Optical densities were measured at 490nm (OD490). Non-specific activity was determined by treating duplicate sample sets with the EPO inhibitor, 3-amino-1,2,4-triazole (2 mM,Sigma), and was always less than 10% of the non-treated samples. Results are expressed as OD490 corrected for background and volume of BALF supernatant retrieved (BALF recovery was 80 ± 5%).

Airway hyperresponsiveness (AHR)

AHR to methacholine challenge was assessed, 24 h following TDI challenge, using a single chamber whole-body plethysmograph (Buxco, Troy, NY). A spontaneously breathing mouse was placed into the main chamber of the plethysmograph, and pressure differences between the main chamber and a reference chamber were recorded. AHR was expressed as enhanced pause (PenH), which correlates with measurement of airway resistance, impedance and intrapleural pressure and is derived from the formula:

$$PenH = [(Te – Tr)/Tr] \times Pef/Pif;$$

where Te = expiration time, Tr = relaxation time, Pef = peak expiratory flow, and Pif = peak inspiratory flow (Schwarze et al., 1999). Mice were placed into the plethysmograph and exposed for 3 min to nebulised PBS followed by 5 min of data collection to establish baseline values. This was followed by increasing concentrations of nebulised methacholine (0–50 mg contained in 1.0 mL of PBS) for 3 min per dose using an AeroSonic ultrasonic nebulizer (DeVilbiss, Somerset, PA). Recordings were taken for 5 min after each nebulisation. The PenH values during each 5 min sequence were averaged and expressed as percentage increase over baseline values following PBS exposure for each methacholine concentration.

Real-time RT-PCR

Tissues were homogenised, and total cellular RNA was extracted using the Qiagen RNeasy kit (Qiagen, Valencia, CA) according to the manufacturer's instructions. One microgram of RNA was reverse-transcribed using random hexamers and 60 U of Superscript II (Life Technologies, Grand Island, NY). Real-time PCR primer/probe sets for murine 18S, IFN_{γ}, IL-4, IL-5, and TNF_{α} were purchased as predeveloped kits from Applied Biosystems (Foster City, CA). Real-time PCR was performed using Taqman Universal Master mix with Amperase in an iCycler (Bio-Rad, Hercules, CA) for 1 cycle at 50 °C for 2 min (degrade carry over using Amperase), and 95 °C for 10 min, followed by 60 cycles at 95 °C for 15 sec and 60 °C for 1 min. The differences in mRNA expression between control and treatment groups were determined by the relative quantification method developed by (Pfaffl, 2001) utilising the threshold cycle (CT) method and real-time PCR efficiencies of the target gene normalized to the housekeeping gene 18S/rRNA.

Statistical analysis

All studies were replicated with representative data shown. For statistical analysis, standard one-way analysis of variance (ANOVA) followed by the Student-Newman-Keuls test was used for multiple group comparisons. Student's two-tailed unpaired t test was used to determine the level of difference between two experimental groups, and $p < 0.05$ was considered a statistically significant difference. For the analyses of RT-PCR data, the fold change from the mean of the control group was calculated for each individual sample (including individual control samples to assess variability in this group centered around one) prior to ANOVA and SNK.

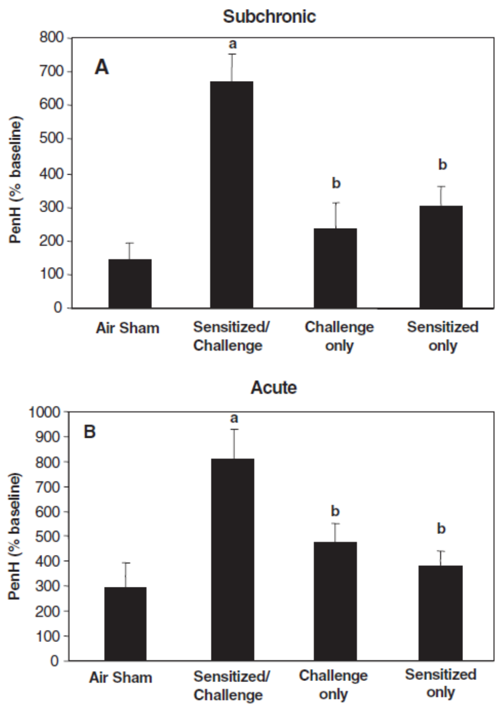

Figure 1: AHR in TDI-exposed mice. Mice which received air only, air sensitised/TDI challenged, TDI sensitised/air challenged, or TDI sensitised/challenged by either subchronic exposure (A) or acute exposure (B) were assessed for nonspecific methacholine reactivity. The change in PenH values in response to 50 mg/mL of inhaled aerosolised methacholine was determined 24 h after challenge and is expressed as percent change from baseline values (aerosolised saline). The PenH baseline values (0.48 ± 0.06) did not differ between treatment groups. Significantly different from a = air sham control group or b = sensitised/challenged group ($p < 0.05$, $n < 5$, mean ± SEM). Taken from (Matheson et al., 2005b).

Results

AHR

The results with respect to Airway Hyperresponsiveness (AHR) are shown in Figure 1 above. Mice exposed to 20 ppb TDI by inhalation for 6 weeks and challenged 14 days later demonstrated a marked increase in AHR to methacholine. A slight increase in AHR to methacholine occurred in the sensitised-only and challenged-only groups, but was not statistically significant. Mice exposed to an acute high dose (500 ppb) of TDI followed 14 days later with 20 ppb challenge also exhibited significant AHR to methacholine challenge compared to controls. No differences in baseline PenH values were observed between treatment groups in the subchronic or acute exposure protocols. Furthermore, mice subchronically exposed to TDI show increased PenH values within 2 h following challenge with TDI, indicating TDI-specific airway responsiveness, an important characteristic of asthma.

For the reporting of the remaining parts of this study, the control group will represent mice that received air exposure for 6 weeks (subchronic) or 2 h (acute) followed by TDI challenge (challenge-only).

Antibodies

The results of the antibody assessment are shown in Figure 2.

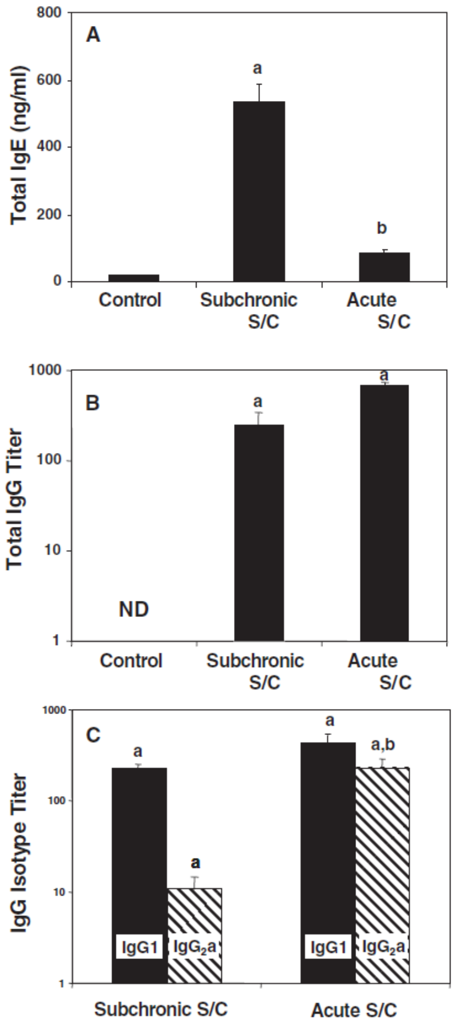

Figure 2: Total serum IgE levels and TDI-specific serum IgG antibody titers. Sera were collected 24 h after TDI challenge from mice that received TDI challenge only (control), subchronic low-dose TDI exposure, or acute high-dose TDI exposure. Total IgE levels (A), TDI-specific IgG antibodies (B), and TDI-specific IgG_1 and IgG_{2a} antibodies (C) are shown. No TDI-specific IgG antibodies were detected in the control group for (C). Significantly different from a = control group or b = subchronic sensitised/challenged group, ($p < 0.05$, n = 5, mean ± SEM). ND = not detected. From (Matheson et al., 2005b).

Twenty-four hours after TDI challenge, blood was collected from control and exposed mice and the serum analysed for total IgE and TDI-specific IgG antibodies. Total serum IgE levels in mice that received subchronic TDI exposure were increased by approximately 10-fold compared to control mice, while IgE levels in serum from mice that received an acute exposure to TDI were comparable to controls. Total IgG TDI-specific antibodies, as well as IgG_1 and IgG_{2a} TDI-specific antibodies, were consistently detected and significantly elevated in both the subchronic low-dose and the acute high-dose exposed groups, compared to undetectable levels found in the control group. In addition, while there were equivalent levels of IgG_1 and IgG_{2a} antibodies in the acute high-dose group, IgG_1-specific antibodies were at least 30-fold higher than IgG_{2a} antibody levels, in subchronically exposed mice. IgG_1 and IgG_{2a} antibodies specific for TDI were not detectable in sera of control mice (not shown).

Markers of inflammation

The pathological changes induced by TDI exposure are summarised in Table 9, followed by an overview of the findings from BALF analysis in Figure 3.

Table 9: Summary of pathological changes induced by TDO exposure, from (Matheson et al., 2005b). Histopathological changes were assessed 48 h after the last TDI inhalation challenge. Values are expressed on a 0–5 scale, with 0 representing no changes, 1 = minimal, 2 = slight/mild, 3 = moderate, 4 = moderately/severe, and 5 = severe. Mean individual severity within a group was calculated by added severity scores of all animals and then dividing that by the total number of animals. a = Significantly different from control group ($p < 0.05$). b= Epithelial changes represent epithelial hyperplasia, epithelial regeneration, and loss of structure. * = Mean ± SEM (n = 5).

Tissue alteration		Control	Subchronic	Acute
Nares				
Exudate		0.2 ± 2[*]	2.5 ± 2[a]	2.2 ± 6[a]
Goblet metaplasia		1.2 ± 0.2	4.2 ± 0.1[a]	4.3 ± 0.2[a]
Inflammation	**Lymphocytes**	0.5 ± 0.2	2.2 ± 0.4[a]	0.5 ± 0.3
	Neutrophils	0.8 ± 0.2	2.7 ± 0.5[a]	1.8 ± 0.6
	Eosinophils	0.4 ± 0.3	2.9 ± 0.5[a]	0.7 ± 0.3
	Epithelial changes	0.2 ± 0.2	2.1 ± 0.1[a]	3.3 ± 0.1[a]
	Hyaline droplet	0.2 ± 0.3	3.1 ± 0.4[a]	2.0 ± 0.2[a]
Lung				
Goblet metaplasia		0	1.9 ± 0.3	2.3 ± 0.7[a]
Inflammation	**Lymphocytes**	0.7 ± 0.3	3.3 ± 0.4[a]	0.8 ± 0.3
	Neutrophils	0	1.9 ± 0.3[a]	0.2 ± 0.2
	Eosinophils	0	3.4 ± 0.3[a]	0.2 ± 0.1
	Macrophages	0	2.4 ± 0.3[a]	1.7 ± 0.2[a]
	Epithelial changes	0	2.4 ± 0.4[a]	1.2 ± 0.3[a]

Airway inflammation is a central feature of the asthmatic response to TDI and is considered a key manifestation of underlying bronchial hyperresponsiveness. Mice subjected to the subchronic TDI exposure regimen presented histological changes in the lungs and nares consistent with an inflammatory response, manifested by neutrophil, lymphocyte, eosinophil, and macrophage infiltration. Tissues at these sites exhibited degenerative cellular changes including loss of cilia, goblet cell metaplasia, septal exudate, hyaline droplet formation, and epithelial hyperplasia. Mice exposed by the acute high-dose exposure regimen exhibited similar histopathology as observed in the subchronic exposure, but fewer inflammatory cells, including eosinophils. Control mice revealed minimal histopathological changes that were contained primarily in the nares.

Total cell numbers in the BALF of mice exposed following the subchronic protocol were increased two-fold compared to the control group. Differential analysis showed that large increases in eosinophils and lymphocytes were responsible for the observed increase in cell recruitment. There was also a significant increase in neutrophil infiltration into the lung, although to a much lesser extent than other inflammatory cells. Macrophages were the predominant cell type in the lung of control mice, representing over 95% of the cells, whereas macrophages decreased to 56% of the total cell population in the subchronically exposed mice following challenge. Mice exposed to the acute high-dose treatment exhibited an 8-fold increase in lymphocyte numbers following challenge, but minimal effects on other inflammatory cells, including eosinophils. Corresponding to the increase in eosinophil numbers, EPO activity in BALF supernatants was significantly elevated in subchronically exposed mice after challenge, while no increase in activity was found in the acute high-dose treated animals.

Cytokines have been implicated in the recruitment of inflammatory cells to the lung and in the pathogenesis of asthma. To determine the effects of TDI on the relative expression of cytokines in the airway, RNA was isolated from the lungs of mice 24 h after challenge, and the levels of IL-4, IL-5, TNF_{α} and IFN_{γ} mRNA were determined by real-time PCR, cf. Figure 4.

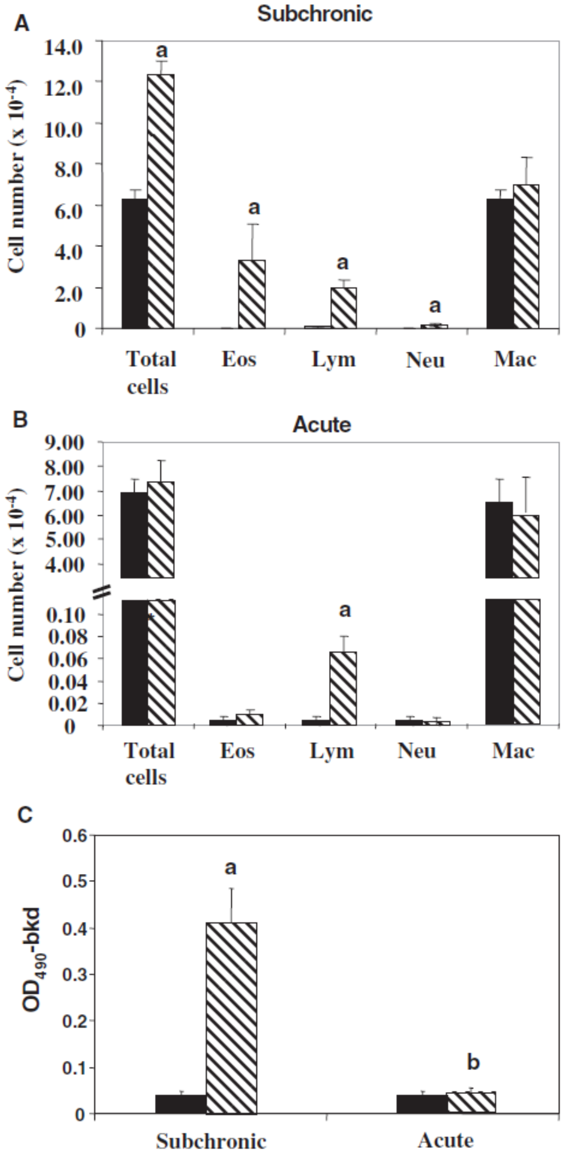

Figure 3: Cellular distribution and EPO activity in bronchoalveolar lavage fluid (BALF). BALF was collected 24 h after TDI challenge, and cytospin preparations were examined for cellular content. Differential cell counts for subchronically exposed mice (A) and acutely exposed mice (B) were determined using light microscopy by evaluation of 300 cells per slide. Data are presented as total cell number for each population in the BALF (Eos = eosinophil; Lym = lymphocyte; Neu = neutrophil; Mac = macrophage). BALF supernatants were measured for eosinophil peroxidase activity (C), and the data are expressed as the optical density at 490 nm after background subtraction (OD490 – bkd). Solid bars represent control group responses, and stripped bars represent TDI sensitised/challenged group responses. Significantly different from a = control group or b = subchronic sensitised/challenged group, ($p < 0.05$, $n = 5$, mean ± SEM). Taken from (Matheson et al., 2005b).

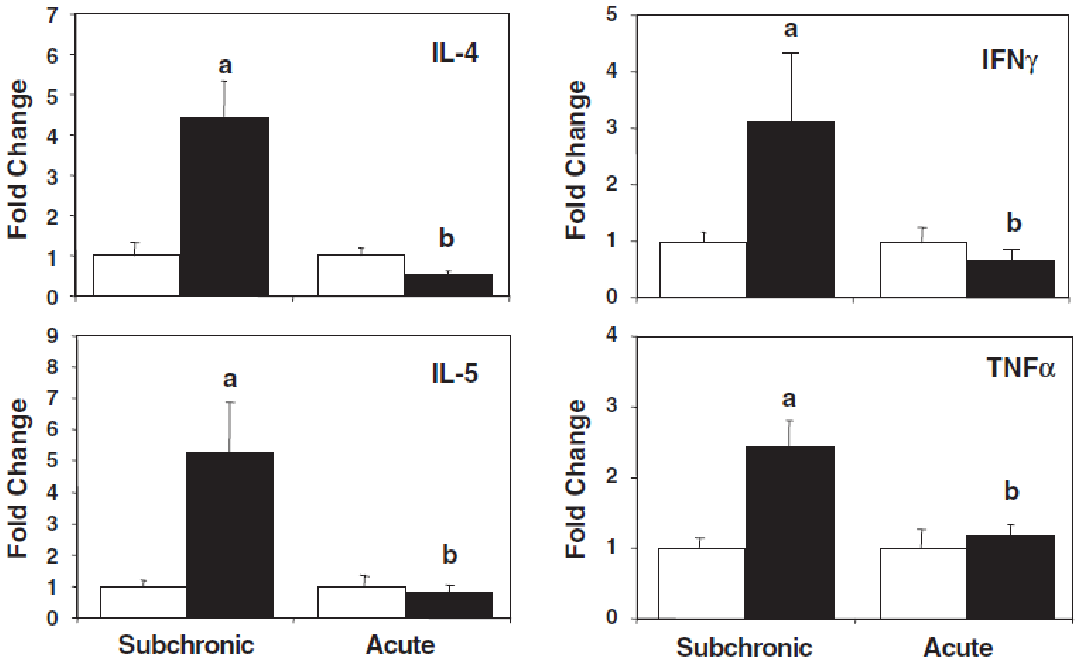

Figure 4: Inflammatory cytokine gene expression in the lungs of TDI-exposed mice. Twenty-four hours following challenge, RNA was isolated from lungs and real-time RT-PCR was performed using IL-4, IL-5, IFN_{γ}, TNF_{α}, or 18s (internal control)-specific primer/probe sets. Cytokine mRNA expression data for subchronic and acute exposure mice are presented as fold change from the respective control group. Open bars represent control group responses, and solid bars represent TDI sensitised/challenged group responses. Significantly different from a = control group or b = subchronic sensitised/challenged group, ($p < 0.05$, n = 4, mean ± SEM).

Compared to the control group, subchronic TDI-exposed mice showed significant elevations in IL-4, IL-5, IFN_{γ} and TNF_{α} mRNA transcripts following TDI challenge. In contrast, no increase in expression of IL-4, IL-5, IFN_{γ} or TNF_{α} was observed in the lungs of mice that received acute TDI exposure.

Transfer experiments

To determine whether specific immunity was involved in the asthmatic response to TDI, adoptive transfer experiments were conducted in which lymphocytes, B cells, or T cells from TDI-exposed mice were transferred into naive recipients. Twenty-four hours following cell transfer, the mice were challenged with 20 ppb TDI, and lung inflammation and airway reactivity were assessed 48 and 24 h later, respectively.

Histological examination of lungs from mice that received lymphocytes from subchronic TDI exposed animals showed slight, diffuse infiltration of lymphocytes and eosinophils following TDI challenge, while those receiving lymphocytes for acute TDI exposed group revealed lymphocyte infiltration but no eosinophils. No lung inflammation was evident after challenge in transfer mice that received lymphocytes from control animals. Naive mice that received either purified lymphocytes, T cells, or B cells from mice that underwent subchronic exposure also displayed significantly increased responsiveness to methacholine 24 h following TDI challenge, when compared to the control group. Recipient mice that received unfractionated lymphocytes from mice in the acute treatment group also showed a significant increase in AHR to methacholine 24 h following TDI challenge, although the magnitude of increase over the control group was about half that observed following total cell transfer from subchronic exposure mice. Adoptive transfer experiments with purified B and T cells from mice that received the acute exposure regimen were not conducted (Figure 5).

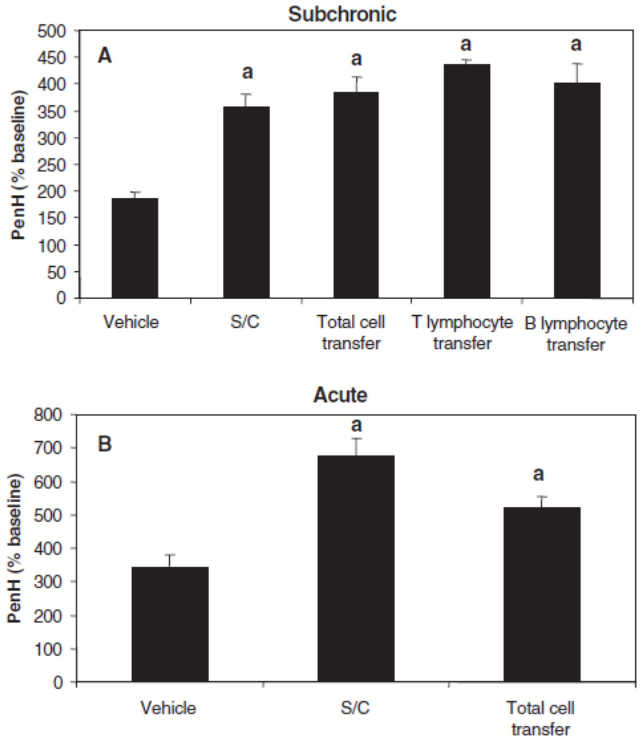

Figure 5: AHR following adoptive transfer with lymphocytes from TDI-exposed mice. Lymphocytes pooled from the auricular lymph nodes and spleens from TDI-subchronically exposed (A) or acutely exposed mice (B) were injected i.v. into naive recipient mice that where challenged by TDI inhalation 24 h later. Twenty-four hours following TDI challenge, mice which received vehicle, total lymphocytes, T lymphocytes, or B lymphocytes, as well as a TDI-exposed positive control group (sensitised/challenged, S/C) were assessed for methacholine reactivity. The change in PenH values in response to 50 mg/mL of inhaled aerosolised methacholine is expressed as percent change from baseline values (aerosolised saline). The PenH baseline values (0.51 ± 0.07) did not differ between treatment groups. a = Significantly different from vehicle control group, $p < 0.05$, n = 5, mean ± SEM. From (Matheson et al., 2005b).

To help determine whether TDI-specific lymphocytes were present in the transfer experiments, lymphocytes from mice that underwent subchronic TDI exposure were adoptively transferred to naive recipients, and 24 h later the recipients were challenged with 25 mL of 1% TDI on the dorsum of the ear. Ear swelling was determined following an additional 24 h. Mice that received unfractionated lymphocytes, B cells, or T cells produced a significant ear swelling response following TDI challenge. Cell proliferation in the draining auricular lymph node was also significantly increased in adoptively transferred mice following TDI ear challenge, although the response following transfer of B cells was minimal compared to T cells. This was evidenced by 20-fold, 8-fold, and 2.4-fold increases in ^{3}H-thymidine uptake in mice receiving total lymphocytes, T lymphocytes, and B lymphocytes, respectively, compared to controls. Transfer of lymphocytes from acutely exposed mice was not performed in these experiments (Figure 6).

To help elucidate the role of humoral immunity in TDI-induced asthma, passive transfer experiments were performed in which serum from mice that had been exposed subchronically and challenged with TDI was administered to naive mice. Histological examination of lungs from mice that received serum from TDI-exposed animals showed minimal diffuse infiltration of lymphocytes and eosinophils 48 h after TDI challenge. No lung inflammation was evident after challenge in transfer mice that were injected with serum from control animals. Twenty-four hours following serum transfer, mice were challenged with TDI by inhalation, and AHR to methacholine was assessed 24 h later. Mice that received non-heated serum from subchronically exposed mice displayed increased AHR to methacholine challenge (50 mg/mL) at 24 h after TDI challenge. Heat inactivation of the serum (56 °C, 4 h), which destroys IgE activity, removed the ability to transfer AHR. Mice injected intradermally with sera (30 mL) from subchronically exposed mice and challenged 24 h later with 1% TDI demonstrated a dermal response, measured as an increase in ear thickness. Heat inactivation of the sera also markedly, but not completely, reduced the dermal response, possibly reflecting the presence of other soluble mediators in the serum that are heat-stable (Figure 7).

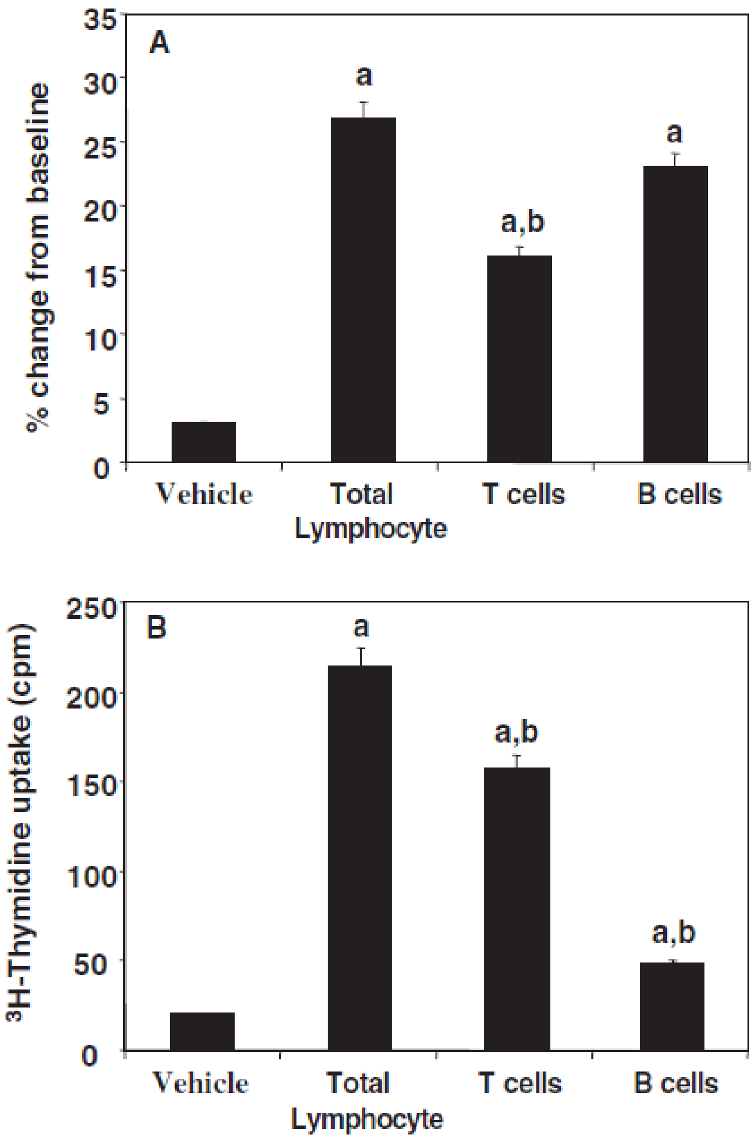

Figure 6: Contact hypersensitivity to TDI following adoptive transfer of lymphocytes from mice subchronically exposed to TDI. Lymphocytes pooled from the auricular lymph nodes and spleens from TDI-exposed mice were injected i.v. into naive recipient mice. Mice were challenged 24 h later with 1% TDI on the dorsum of the right ear, and after an additional 24 h, contact hypersensitivity responses were measured as a function of challenge-induced increases in ear thickness (A) and ^{3}H-thymidine uptake in the draining auricular lymph nodes (B). Significantly different from a = vehicle control group or b = total lymphocyte transfer group, ($p = 0.05$, $n = 4$, mean ± SEM). From (Matheson et al., 2005b).

The role of antibody in TDI-induced asthma was further explored using FcErIg transgenic mice, which lack the g chain subunit of the FceRI, FcgRIII, and FcgRI receptors and, thus, do not mount functional IgG and IgE immune responses. Transgenic mice were exposed to TDI by subchronic inhalation, and methacholine reactivity was assessed at 24 h following TDI challenge. Increased AHR in transgenic mice was similar to the controls. Changes in lung cytokine mRNA expression were also examined in FcErIg transgenic mice. In contrast to the sensitized/challenged wildtype group, the levels of the asthma-associated cytokines IL-4, IL-5, IFN_g and TNF_a in the subchronically exposed FcErIg transgenic mice were not increased (Figure 8).

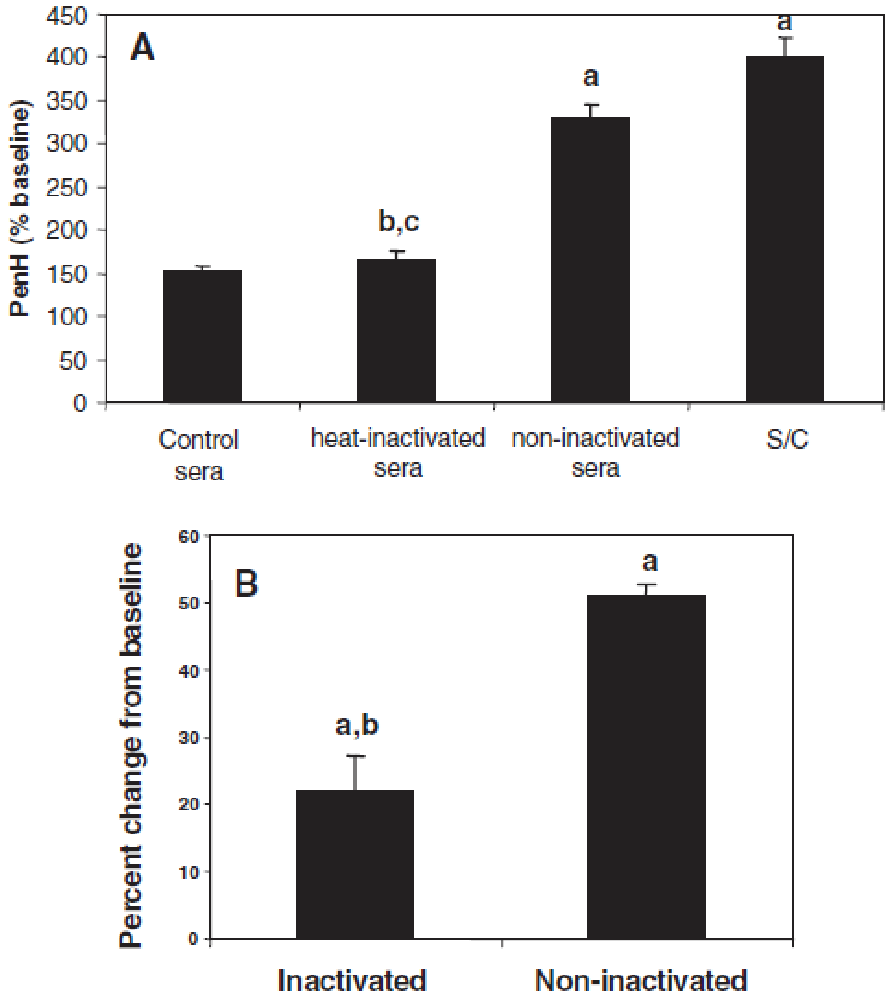

Figure 7: AHR following passive transfer of TDI immune serum. Sera pooled from TDI subchronically exposed mice was injected i.v. into naive recipient mice. (A) Twenty-four hours later mice were challenged with TDI (20 ppb via inhalation route for 1 h) and 24 h post-inhalation challenge, mice which received control sera, heat-inactivated TDI sera, noninactivated TDI sera, or TDI subchronic sensitised/challenged (S/C, positive control) were assessed for methacholine reactivity. The change in PenH values in response to 50 mg/mL of inhaled aerosolised methacholine is expressed as percent change from baseline values (aerosolised saline). The PenH baseline values (0.45 ± 0.04) did not differ between treatment groups. (B) Heat-inactivated or non-inactivated pooled serum from TDI subchronically exposed mice was injected intradermally into the dorsum of the right ear of naive recipient mice. Twenty-four hours following transfer, mice were challenged with 1% TDI on the same ear, and responses were measured as a function of challenge-induced increases in ear thickness 24 h post-challenge. Data are presented as percent change from pre-challenge ear thickness of the right ear. Significantly different from a = control serum treated group, b = non-inactivated treated serum group, or c = subchronic sensitised/challenged group, ($p < 0.05$, n = 5, mean ± SEM). The response to control sera was compared to that of normal mouse sera, and no difference was observed (data not shown). From (Matheson et al., 2005b).

Conclusion of the authors

In conclusion, a mouse model is described that demonstrates low-level subchronic TDI inhalation induces pathology, consistent with allergic asthma, manifested by airway inflammation, lung eosinophilia, increased AHR, asthma associated histopathology, Th cytokine expression, elevated serum IgE, and TDI-specific antibodies. Asthmatic symptoms also occur following high-dose, acute exposure, but the response is less robust, failing to demonstrate eosinophilia, elevated serum IgE levels, or Th cytokines. Evidence is also presented that, like allergic asthma, TDI asthma following subchronic exposure, while associated with a T_H2 response involving IgE antibodies, also involves T_H1 responses.

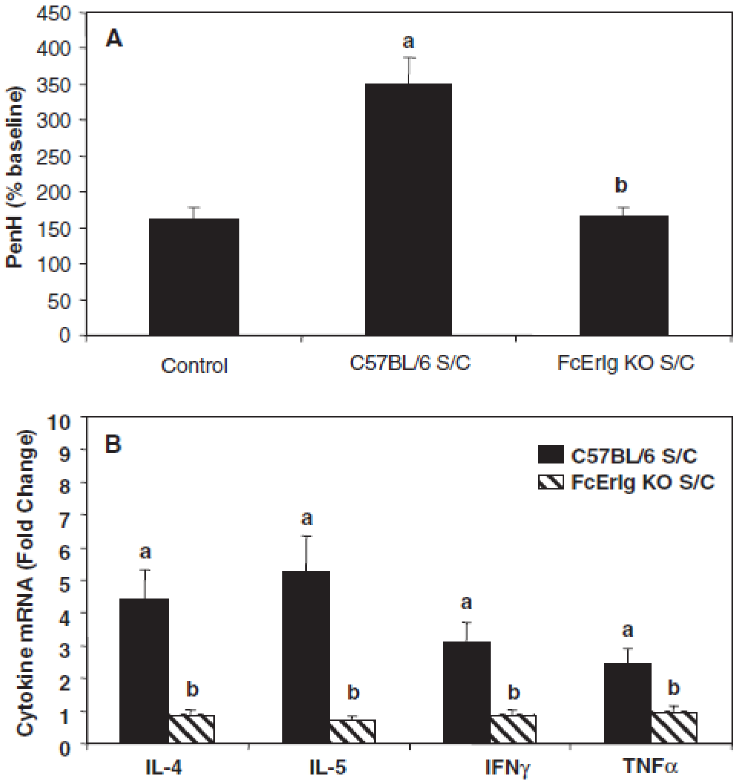

Figure 8: AHR and lung cytokine expression in mice lacking Fc-e and Fc-g (FcErIg) receptors after subchronic exposure to TDI. (A) Twenty-four hours following TDI inhalation challenge, control mice, FcErIg knockout S/C mice, or TDI-subchronically exposed C57BL/6 S/C mice were assessed for methacholine reactivity. The change in PenH values in response to 50 mg/mL of inhaled aerosolised methacholine was determined 24 h after challenge and is expressed as percent change from baseline values (aerosolised saline). The PenH baseline values (0.42 ± 0.08) did not differ between treatment groups. (B) Twenty-four hours following TDI challenge, mice were sacrificed, RNA was isolated from the lungs, and real-time RT-PCR was performed using IL-4, IL-5, IFN-γ, TNFα, and 18S-specific primer/probe sets. Data are presented as fold changes from the corresponding control strain. Significantly different from a = control group or b = wild-type sensitised/challenged group, ($p < 0.05$, n = 5, mean ± SEM). S/C = TDI sensitised/ challenged C57BL/6 mice from subchronic exposure. From (Matheson et al., 2005b).

1.1.2.3 Hoymann et al., 1995

Summary as provided by the lead registrant for MDI (the full study report was not available to the DS).

Study reference:

Hoymann H.G., Buschmann J., and Heinrich U. (1995): Untersuchungen zur chronischen Toxizität/ Kanzerogenität von 4,4'-Methylendiphenyl-Diisocyanat (MDI) [Studies on the chronic toxicity/carcinogenicity of 4,4'-methylenediphenyl-diisocyanate (MDI)]. Forschungsbericht 116 06 084, date: 1995-09-01. Fraunhofer-Institut für Toxikologie und Aerosolforschung. Umweltbundesamt (UBA)

Only a IUCLID summary of this study was available from which only the details relevant for RS are reproduced below. Details are confined to findings.

Test type:

Combined chronic/carcinogenicity test claimed to be similar to OECD 453, but with only female animals exposed and exposure limited to 17 h/d. GLP claimed.

Test substance:

Monomeric 4,4'-methylenediphenyl diisocyanate (Desmodur 44 M Schuppen from Bayer AG, Leverkusen); 13 batches were tested (purity: > 99.5 %)

Test animals:

Rat, Crl:[WI]BR Wistar, female. At the start of the study the animals were approximately 10 weeks old. Acclimation: approx. 2 weeks. Origin: Charles River Wiga GmbH, Sulzfeld. 80 females per dose; at each dose level there were additional 80 rats per group in satellite groups for:

- chronic toxicity over 12 months (20 animals),
- lung function over 20 months (12 animals),
- lung clearance over 20 months (8 animals),
- bronchoalveolar lavage, biochemistry over 3 months + 1 week recovery (20 animals), and
- bronchoalveolar lavage, biochemistry over 12 months + 1 week recovery (20 animals).

Administration/exposure:

Choice of the exposure concentrations was done after a range-finding test (90-day study at 0.3, 1 und 3 mg/m³, under exposure regime of ca. 18 hours/day, 5 days/week), where a no observed effect concentration was derived (NOEC: 0.3 mg/m^3), based on substance-related effects seen in the highest and to some extent also in the mid-dose group. MDI aerosol was generated using an evaporation-condensation technique. The rats were exposed via whole-body exposure to concentrations of 0-0.2-0.7-2.1 mg/m^3, 17 h/d, 5 d/wk, for up to two years in 6 m^3 stainless steel inhalation chambers (horizontal air flow, renewal rate: approx. 15-fold per hour). Since the vapour saturation of MDI at 23°C is about 0.1 mg/m^3, a part of the exposure was as vapour. Monitoring of total MDI was performed by gravimetrically calibrated, light scattering aerosol sensors. Concentrations of monomeric MDI in the inhalation chamber were measured with HPLC. The median mass aerodynamic diameters (in µm) were 1.03, 1.03, and 1.06, respectively. Controls: yes, sham-exposed.

Examinations:

Clinical signs:

All animals were observed for clinical signs at least once a day; if clinical signs were present, the animals were further examined; animals in bad condition were killed and organs put in formalin.

Organs examined at necropsy:

Macroscopic examination: full pathological examination is done on the surviving rats of the chronic tox test killed at 12 months exposure (satellite groups) and at 12 months resp. 24 months (animals with number 101-120 resp. 1-80) of the carcinogen test. Following organs are preserved in 10 % neutral buffered formalin solution: all organs/tissues that are macroscopically changed, brains, pituitary, thyroid, thymus, larynx and laryngopharynx, trachea, lungs, heart, aorta, pancreas, liver, kidney, adrenals, periferal nerve, sternum, femur and knee, vertebrae, tongue, lymph nodes (submandibular and mesenteric), mediastinal lymph nodes, nose, sinus, eyes/Harderian glands; lacrimal glands (extraorbitale), ovaries, uterus and vagina, mammary, skin, oesophgus, stomach, duodenum, jejunum, ileum, caecum, colon, rectum, urinary bladder, muscles, pancreas, mesenterium. Lungs (incl trachea), under +/- 20 cm water pressure, are preserved in formaline solution.

Organ weights: are performed on the animals of the satellite group used for chronic tox test after 12 months of exposure: in 10 animals/ group: fresh weights of brain, liver, kidneys and adrenals and ovaries. Also the relative organ weights are calculated (vs. the body weight at the end of the test). This examination was not performed in rats after 24 months of testing due to increased mortality and the number of surviving animals being too limited to allow any firm conclusions to be drawn. In the satellite groups used to examine BAL (10 animals/ group) at the end of the exposure time as well as on the remaining 10 animals/group after recovery (=after 20 months: in surviving animals of the 20 animals/group at end of the test) terminal body weights and fresh weight on lungs (incl trachea) as well as the relative lung weight are calculated.

Microscopic examination (light microscopy) was done for all animals of the control group and the high dose group of the carcinogenicity test and the chronic tox after 12 months, on above tissues/organs after haematoxylin-eosin staining (Lilly-Meyer). In case of substance related pathological findings found in these groups, all corresponding organs (respiratory tract) of all other animals of low and mid-dose groups are examined. Moreover all organs with tumor-like or similar modifications were histologically examined. Peer review of the lung examinations (review examination by an external pathologist by Prof. Dr. D.L. Dungworth, University of California, Davis, USA. Data record and statistical treatment of the pathological findings was done using the PLACES program.

Other examinations:

- lung function: on rats under narcosis, with non-invasive method. After 6, 12 and 17 months identical tests were done on the same rats (of the satellite groups). a) Whole-body plethysmography and parameter on spontaneous breathing. b) Forced Expiration c) Lung volume and elasticity d) N-exchange test: homogenity of ventilation e) CO-diffusion test: diffusion,
- bronchoalveolar lavage (BAL): Biochemical and cytological parameter of lung lavage, b) measurement of surface tension,
- lung clearance, and
- investigations on MDI-metabolism: in blood and urine.

Statistics:

Differences between test and control groups are judged statistically significant at level $p<0.05$. Body weight and food consumption, absolute and relative organ weight and hematological/biochemical data, BAL, clearance and lung function data are checked for difference between groups by variance analysis. If statistical difference was found between group means, the mean of the test group was compared to the mean of the control by t-test (lung function) or adapted t-test (Dunnett-test). The Wilcoxon test was used for surfactant data. Qualitative and semi-quantitative data (histopathology) are analysed by Fisher-test.

Any other information on materials and methods incl. tables:

The photometrically determined chamber concentrations were 0.23, 0.70 and 2.05 mg/m^3, with standard deviations of 0.06, 0.17 and 0.37 mg/m^3, respectively. The fraction of the total MDI concentration present as monomeric 4,4'-MDI was 43%, 79% and 85%, respectively, for the low, mid and high exposure groups. The fraction of the total MDI concentration present as monomeric 4,4'-MDI was 43%, 79%, and 85%, respectively, for the low, mid and high exposure groups. The fraction of the total MDI concentration present as monomeric 4,4'-MDI was 43%, 79%, and 85%, respectively, for the low, mid and high exposure groups.

Results and discussion:

Mortality: decreased survival time was seen in all groups (including controls). This was due to the earlier onset of age related changes e.g. tumours of pituitary and mammary gland. The cause of this finding could not be foreseen at the start of the test nor can it be clarified. In the carcinogenicity test: No significant differences occurred between the test groups and the controls. After 17-18 months exposure (i.e. 19-20 months age) cumulative mortality was 50%. Compared to internal and external historical data (1984-1988) on the same rat species, this represents a real decrease in survival time. After 17 months of exposure the weight differences from low, mid and high dose groups compared to controls were -6.7%; -7.9% and -11.3%. However it should be noted that at day 0 the weights of mid and high dose group were 2.4 and 2.2% lower.

Body weight: since 4.5 months of testing, the mean weight of the animals in the mid- and low dose groups were significantly decreased compared to the control group.

Organ weights: Lungs: relative fresh weights (normalised to body weight) for lungs are increased after 3, 12 and 20 months exposure. After 3 months: significantly increased weights in all test groups. After 12 and 20 months these differences are only present at the highest dose group. After 1 week recovery (clean air) following 3 months exposure, a recovery effect is seen in the low and mid dose. However, in the high dose group animals the lung weight remains sign increased. Histopathological changes corroborate with this finding. Other organs: no significant difference are seen between the test and control groups

Gross pathology: with exception of the changes as described under histopathological changes, no substance related changes could be found

Histopathology: I. After 12 months of exposure (satellite-groups): Non-neoplastic changes: Exposure related pathological changes were only found in the nose, lungs and lung associated lymph nodes (LALN). Nose: Very low to low graded (multi)focal degeneration of the olfactory epithelium: in 5/15 animals of the high dose group; in 1/19 animals of the mid dose group. These changes were absent in the low and control group. Statistically different were control and high dose group. Other changes were seen but these were not statistically significant from the controls. After 12 months MDI exposure: MDA-DNA adducts were found in olfactory nose epithelium, however only in marginal amount. Remark: The proof of MDA-DNA adducts is possibly feigned by the strong protein binding. The toxicological relevance of this finding is doubtful since MDI leads only in high concentrations to degeneration of the olfactory epithelium (Greim H (ed.) 2008, in: Occupational Toxicants - Critical data evaluation for MAK values and classification of carcinogens, Wiley-VCH, Weinheim, Vol. 14). Lungs: Statistically significant multifocal to diffuse interstitial (septal) fibrosis in all exposure groups. Slight to moderate interstitial fibrosis in mid and high dose group: present in resp. 18/19 animals and 15/15 animals (diff. not statistically significant). In the low dose group: 6/19. Moderate (multi)focal bronchiole-alveolar hyperplasia: higher frequency in mid and high dose groups. Focal alveolar hyperplasia (Type II cells especially): only in exposed groups (1 animal in low and in mid dose; 3 in the high dose). Not significant different but presumably related to exposure. Alveolar accumulation of macrophages with inclusion of particles in low amount and dose related frequency: only present in groups exposed to the test substance (statistically different compared to control: low dose: 8/19; mid: 16/19 and high dose: 15/15 animals). Epithelium associated giant cells of Langhans: difference very significant in mid and high dose groups. Low to moderate interstitial mononuclear cell infiltration in control to high dose animals: resp. 2/18; 5/19; 18/19 and 13/15. In the BAL there were after 3 and 12 months in the highest dose; increased macrophages, lymphocytes numbers; after 20 months increased number of lymphocytes. At no point in time was there a change in the number of granulocytes. Lung associated lymph nodes (LALN): Exposure related multifocal accumulation of particle bearing macrophages: in the mid (16/19) and high (6/14) dose group (statistically different from control). Slight reactive hyperplasia of the lymphoid tissue associated with macrophage accumulation: dose dependent increase in incidence. Other organs: Exposure related changes could not be detected.

Histopathology: II. After 24 months of exposure (carcinogenicity test): Lungs: A dose related neoplastic effect was only seen in the lungs. In 1 animal of the high dose group: bronchiole-alveolar adenoma built of dysplastic alveolar cells (type II pneumocytes). Further: dose dependent (multi)focal high grade dysplastic alveolar hyperplasia. Exposure related changes could only be found in the nose, larynx, lungs and lung-associated lymph nodes. Nose (only examined in control and high dose group): (Multi)focal, in general moderate squamous metaplasia, mainly in the proximity of the olfactory epithelium (in high dose significantly higher than in control: 16/80 vs 5/80). (Multi)focal generally moderate Becker cell hyperplasia (50/80 vs 33/80) and inflammatory cell infiltration of the mucosa (29/80 vs 10/80). Other changes, non significant but obviously dose related were: metaplasia of the respiratory epithelium, degeneration, erosion, respiratory and/or olfactory epithelium. Larynx (only examined in controls and high dose group): Slight multi(focal) squamous metaplasia significantly higher (13/79 vs 1/80). Focal hyperkeratosis (in the area of the epiglottis) and inflammatory infiltration of the mucosa (however non significant). Lungs: Alveolar cell hyperplasia: in frequency and severity significant difference between mid and high dose compared to controls. In the following incidences and severity are described for the 3 dose groups (number of animals with grade of the effect: very slight, slight, moderate, high; total animals displaying these changes): Low dose: 1/80; 4/80; 2/80; 1/80; 8/80, Mid dose: 0/80; 5/80; 5/80; 2/80; 12/80, High dose: 0/80; 6/80; 8/80; 7/80; 21/80. Alveolar bronchiolisation: (Multi)focal bronchiole-alveolar hyperplasia: is significantly higher in mid and high dose group (frequency in low; mid, high dose and control: 3/80; 14/80; 41/80; 3/80). The grading of this finding appeared to be dose related. The moderate and high grade hyperplasia only occurred in resp 5 and 2 animals of the high dose exclusively. Interstitial and peribronchiolar fibrosis: In all MDI exposed groups: statistically highly ($p<0.001$) significant compared to control (low, mid, high dose; control: 51/80; 73/80; 77/80; 4/80). Also the severity was significant difference in the different exposure groups: generally very slight (minimal) in low dose; mainly slight and slight to moderate in the high dose group. Other statistically significant dose dependent effects in lungs: Focal to multifocal alveolar accumulations of particle-laden (MDI?) macrophages: in very slight to moderate grade in all exposure groups: 52/80; 70/80 and 78/80 (highly sign diff with controls). Identity of the inclusion could not be defined via light microscopy.

In BAL: after 3 and 12 months of exposure increased number of macrophages and lymphocytes were seen; after 20 months only increased number of lymphocytes. Interstitial mononuclear cell infiltration (mainly low grade): Statistically significant in all exposure groups: number of animals with this finding in resp low; mid, high dose and controls were: 24/80; 48/80; 73/80 and 11/80. Accumulation of hemosiderin pigmented macrophages: from low to high grade dose dependent significantly increased in all exposure groups compared to controls: numbers for low, mid, high dose and control: 6/80; 9/80; 14/80 and 0/80. Small focal to multifocal cholesterol granulomas: in the high dose group: 11/80 vs 0/80 in controls. In the other groups: 4/80 low dose and 1/80 in the mid dose group. Focal osseous metaplasias: Incidence: significantly higher in high dose group vs control (resp. 11/80 and 1/80). In the low and mid dose group resp: 6/80 and 4/80. Lung associated lymph nodes (LALN; only examined in control and high dose group): Accumulation of macrophages with cytoplasmatic inclusions were seen in 68/80 high dose animals (highly significant differences with control were no such changes were observed). In addition, slight to moderate reactive lymphoid hyperplasia was seen, more frequent in high dose (13/80 vs control 6/80). Other organs: Exposure related changes could not be detected. Lung function tests: 1. Significant increased flow resistance in the small, peripheral air tracts in highest dose after 6 months. After 12 and 17 months also detected in the mid and low dose detected (cfr FEV0.1; FEF50 and FEF25). 2. Significantly reduced vital to total lung volume and elasticity of the lung tissue in the high dose already after 6 months (restrictive lung changes). After 12 resp 17 months increased incidence and finally also in the mid dose group and marginally in the low dose group. 3. Positive N-exchange test (indication of increased non-homogenity of the alveolar respiration) after 17 months in the mid and more expressed in the high dose group (already as a trend to be seen after 12 months). 4. Positive CO-diffusion test after 12 and 17 months : particularly in the high dose, less in the mid and marginally in the low dose group (indicating impairment of the diffusion through the alveolar-capillary membrane).

BAL findings: Changes in biochemical lavage parameters (increased lactate dehydrogenase, beta-glucuronidase, total protein, gamma-glutamyl transferase, hydroxyproline concentration, phospholipid concentration; indications of damage to the cell membrane vessel endothelium, cell necrosis, increased collagen metabolism) occurred generally already after 3 months exposure and increased after 12 and 20 months. After 1 week recovery with clean air, these findings seemed partially reversible. Increased concentration of surfactant-phospholipid were found in the mid and high dose groups. Functionally: a slight decrease in 'specific' surface activity of the phospholipid standardised surfact sample is observed in the high dose group (increased surface tension as measured by surfactometer). Increased lymphocyte concentration was seen after 3, 12 and 20 months (partially reversible after 1 week recovery with clean air). Increased number of macrophages after 3 months. The increased lung weights especially in the high dose group were still increased after 1 week recovery. This indicates chronic lung changes that were confirmed by the histopathological findings. Examination of the lung clearance (alveolar lung wash): After 6 months in the high dose group nearly doubled clearance half time compared to control. After 18 months this effect was not detectable anymore. Examination of blood and urine: Hemoglobin adducts and MDA urine concentrations were found in all MDI groups after 3 and 12 months exposure. A steady-state was observed after 3 months exposure.

Conclusion of the authors

In a long-term inhalation study over a maximum of 24 months including satellite groups with 3, 12, and 20-month exposure, the chronic toxicity and carcinogenicity of monomeric methylene diphenyl diisocyanate (MDI) were investigated. Female Wistar rats were exposed in 6 m³ inhalation chambers for 17 hours/day, 5 days/week to 0.23, 0.70 and 2.05 mg/m³ MDI in aerosol form, a control group was kept in clean air. Essentially, a dose-dependent impairment of the lung function in the sense of an obstructive-restrictive malfunction with diffusion disorder, increased lung weights, an inflammatory reaction with increased appearance of lymphocytes (but not of granulocytes) in the lung in the high dose group as a sign of specific stimulation of the immune system by MDI, an intermediately retarded lung clearance in the high dose group as well as dose-dependent interstitial and peribronchiolar fibrosis, alveolar bronchiolisations and a proliferation of the alveolar epithelium, which was classified as preneoplastic, as well as a bronchioloalveolar adenoma were ascertained. The LOAEC for the female rat was 0.23 mg/m^3 after long-term inhalation of 4,4'-MDI aerosols.

2 REFERENCES

Adams W.G.F. (1975): Long-term effects on the health of men engaged in the manufacture of tolylene di-isocyanate. British Journal of Industrial Medicine 32 (1), 72-78. DOI: 10.1136/oem.32.1.72 (last accessed 2016-09-19)

Akbar-Khanzadeh F. and Rivas R.D. (1996): Exposure to isocyanates and organic solvents, and pulmonary-function changes in workers in a polyurethane molding process. Journal of Occupational and Environmental Medicine 38 (12), 1205-1212

Akimoto T., Tamura N., Uchida K., Dambara T., Nukiwa T., and Kira S. (1992): A case of hypersensitivity pneumonitis due to isocyanate exposure showing progression even two months after removal of the antigen. Nihon Kyobu Shikkan Gakkai Zasshi 30 (3), 458-463. DOI: 10.11389/jjrs1963.30.458 (last accessed 2016-09-19)

Alexandersson R., Hedenstierna G., Plato N., and Kolmodin-Hedman B. (1987): Exposure, lung function, and symptoms in car painters exposed to hexamethylendiisocyanate and biuret modified hexamethylendiisocyanate. Archives of Environmental Health 42 (6), 367-373. DOI: 10.1080/00039896.1987.9934361 (last accessed 2016-09-28)

Alexandersson R., Hedenstierna G., Randma E., Rosen G., Swenson A., and Tornling G. (1985): Symptoms and lung function in low-exposure to TDI by polyurethane foam manufacturing. International Archives of Occupational and Environmental Health 55 (2), 149-157. DOI: 10.1007/BF00378377 (last accessed 2016-09-28)

Aoyama K., Huang J., Ueda A., and Matsushita T. (1994): Provocation of respiratory allergy in guinea pigs following inhalation of free toluene diisocyanate. Archives of Environmental Contamination and Toxicology 26 (3), 403-407. DOI: 10.1007/BF00203570 (last accessed 2016-09-19)

Arts J.H.E., de Jong W.H., van Triel J.J., Schijf M.A., de Klerk A., van Loveren H., and Kuper C.F. (2008): The respiratory local lymph node assay as a tool to study respiratory sensitizers. Toxicological Sciences 106 (2), 423-434. DOI: 10.1093/toxsci/kfn199 (last accessed 2016-09-19)

Aul D.J., Bhaumik A., Kennedy A.L., Brown W.E., Lesage J., and Malo J.L. (1999): Specific IgG response to monomeric and polymeric diphenylmethane diisocyanate conjugates in subjects with respiratory reactions to isocyanates. Journal of Allergy and Clinical Immunology 103 (5 Pt 1), 749-755. DOI: 10.1016/S0091-6749(99)70415-X (last accessed 2016-09-28)

Ban M., Hettich D., Goutet M., and Bonnet P. (1997): TDI inhalation in guinea-pigs involves migration of dendritic cells. Toxicology Letters 93 (2-3), 185-194. DOI: 10.1016/S0378-4274(97)00091-X

Ban M., Morel G., Langonné I., Huguet N., Pépin E., and Binet S. (2006): TDI can induce respiratory allergy with Th2-dominated response in mice. Toxicology 218 (1), 39-47. DOI: 10.1016/j.tox.2005.09.013 (last accessed 2016-09-19)

Banks D.E., Barkman H.W., Jr., Butcher B.T., Hammad Y.Y., Rando R.J., Glindmeyer H.W., 3rd, Jones R.N., and Weill H. (1986): Absence of hyperresponsiveness to methacholine in a worker with methylene diphenyl diisocyanate (MDI)-induced asthma. CHEST 89 (3), 389-393. DOI: 10.1378/chest.89.3.389 (last accessed 2016-09-19)

Banks D.E. and Rando R.J. (1988): Recurrent asthma induced by toluene diisocyanate. Thorax 43 (8), 660-662. DOI: 10.1136/thx.43.8.660 (last accessed 2016-09-28)

Banks D.E., Rando R.J., and Barkman H.W. (1990): Persistence of toluene diisocyanate-induced asthma despite negligible workplace exposures. CHEST 97 (1), 121-125. DOI: doi:10.1378/chest.97.1.121 (last accessed 2016-09-19)

Banks D.E., Sastre J., Butcher B.T., Ellis E., Rando R.J., Barkman H.W., Jr., Hammad Y.Y., Glindmeyer H.W., and Weill H. (1989): Role of inhalation challenge testing in the diagnosis of isocyanate-induced asthma. CHEST 95 (2), 414-423. DOI: 10.1378/chest.95.2.414 (last accessed 2016-09-19)

BASF (2009): IPDI Trimer - subchronic 90-day inhalation and lung toxicity study in Wistar rats - dust aerosol exposure. Report no. 99I0357/06026, date: 2009-02-19. BASF SE, Experimental Toxicology and Ecology. Bayer MaterialScience AG, BASF SE, Evonik Degussa GmbH, PERSTORP France, unpublished

Basketter D.A. and Gerberick G.F. (1996): An interlaboratory evaluation of the Buehler test for the identification and classification of skin sensitizers. Contact Dermatitis 35 (3), 146-151. DOI: 10.1111/j.1600-0536.1996.tb02332.x (last accessed 2016-09-19)

Baur X. (1995): Hypersensitivity pneumonitis (extrinsic allergic alveolitis) induced by isocyanates. Journal of Allergy and Clinical Immunology 95 (5), 1004-1010. DOI: 10.1016/S0091-6749(95)70101-X (last accessed 2016-09-19)

Baur X., Dewair M., and Römmelt H. (1984): Acute airway obstruction followed by hypersensitivity pneumonitis in an isocyanate (MDI) worker. Journal of Occupational Medicine 26 (4), 285-287

Bayer (1968): Isophorondiisocyanat - toxikologische Untersuchungen [Isophorone diisocyanate - toxicological studies]. Report no. 908, date: 1968-07-22. Bayer AG, Department of Toxicology. Bayer MaterialScience AG, unpublished

Bayer (1970): Desmodur H: Toxikologische Untersuchungen [Desmodur H: Toxicologial studies]. Report no. 2146, date: 1970-07-08. Bayer AG, Department of Toxicology. Bayer MaterialScience AG, unpublished

Bayer (1983): Hexamethylen-1,6-diisocyanat (HDI): Untersuchungen zur sensibilisierenden Wirkung an der Meerschweinchenhaut [Hexamethylene-1,6-diisocyanate (HDI): Study of a sensitising effect in guinea pig skin]. Report no. 11703, date: 1983-04-08. Bayer AG, Department of Toxicology. Bayer MaterialScience AG, unpublished

Bayer (1984a): 4,4'-Diisocyanato-dicyclohexylmethan (H-MDI), Untersuchungen zur sensibilisierenden Wirkung an der Meerschweinchenhaut (modif. Maximierungstest mit nur intrakutaner Induktion) [4,4'-Diisocyanato-dicyclohexylmethane (H-MDI) - study on a sensitising effect in guinea-pig skin (modif. "maximisation test" with only intracutaneous induction)]. Report no. 13039, date: 1984-11-15. Bayer AG, Department of Toxicology. Bayer MaterialScience AG, unpublished

Bayer (1984b): Isophorondiisocyanat (IPDI) - Untersuchungen zur sensibilisierenden Wirkung an der Meerschweinchenhaut (modif. "Maximierungstest" mit nur intrakutaner Induktion) [Isophorone diisocyanate (IPDI) - study of a sensitising effect in guinea-pig skin (modif. "maximisation test" with only intracutaneous induction)]. Report no. 13041, date: 1984-11-15. Bayer AG, Department of Toxicology. Bayer MaterialScience AG, unpublished

Bayer (1985): 4,4'-Diisocyanato-dicyclohexylmethan (H-MDI), Prüfung auf sensibilisierende Wirkung an der Meerschweinchenhaut (Maximierungstest nach Magnusson/Kligman) [4,4'-Diisocyanato-dicyclohexylmethane (H-MDI), test for a sensitising effect in guinea-pig skin (maximisation test according to Magnusson/Kligman)]. Report no. 13787, date: 1985-08-29. Bayer AG, Department of Toxicology. Bayer MaterialScience AG, unpublished

Bayer (1993): Isophorondiisocyanat - Untersuchungen auf hautsensibilisierende Wirkung bei Meerschweinchen (Maximierungstest nach Magnusson und Kligman) [Isophorone diisocyanate - studies of a skin sensitising effect in guinea-pigs (maximisation test according to Magnusson and Kligman)]. Report no. 22645, company study no. T 1055173, date: 1993-11-04. Bayer AG, Department of Toxicology. Bayer MaterialScience AG, unpublished

Bayer (1994): MDI (Desmodur®44M) Evaluation of respiratory sensitization in guinea-pigs following intradermal induction and MDI-challenge. Report no. T7055340, date: 1994-11-18. Bayer AG, Department of Toxicology. International Isocyanate Institute, unpublished

Bayer (1995a): Desmodur W, pilot study for lung sensitization in Guinea-pigs following intradermal induction. Report no. 24199, date: 1995-07-26. Bayer AG, Department of Toxicology. Bayer MaterialScience AG, unpublished

Bayer (1995b): Diphenylmethane-4,4'-diisocyanate (MDI-monomer). Evaluation of respiratory sensitization in guinea-pigs following brief high-level inhalation induction exposure and challenge with ramped MDI concentrations. Report no. T1058323, III project 121-EU-MTX, III Report 11184, date: 1995-05-30. Bayer AG, Department of Toxicology. International Isocyanate Institute, unpublished

Bayer (1996a): IPDI (Isophorondiisocyanat), evaluation of respiratory sensitization in guinea-pigs following intradermal induction. Report no. 24967, T5055474, date: 1996-04-04. Bayer AG, Department of Toxicology. Bayer MaterialScience AG, unpublished

Bayer (1996b): Vestanat T 1890/100 - study for lung sensitization in guinea-pigs following intradermal or inhalation induction. Report no. 24843, company study no. T6059147, date: 1996-02-28. Bayer AG, Department of Toxicology. Bayer MaterialScience AG, Evonik Degussa GmbH, unpublished

Bayer (2003a): Desmodur Z 4470 MPA/X - local lymph node assay in mice (LLNA/IMDS). Report no. AT00334, date: 2003-03-26. Bayer AG, Department of Toxicology. Bayer MaterialScience AG, unpublished

Bayer (2003b): Monomeric MDI: Lethality to rats following one hour inhalation of an inhalable aerosol. III report 11472, III project 216-AM-MTX, date: 2003-01-15. Bayer AG, PH-PD-P Healthcare Toxicology. International Isocyanate Institute, unpublished

Bayer (2008a): 90-Day inhalation study with a 4-week recovery period in Wistar rats. Report no. AT04587, company study no. T7076969, date: 2008-05-21. Bayer HealthCare AG. Bayer MaterialScience AG, Evonik Degussa GmbH, unpublished

Bayer (2008b): Isophorone diisocyanate (IPDI) 90-day inhalation study with a 4-week recovery period in Wistar rats. Report no. AT04738, company study no.: T6077598, date: 2008-07-31. Bayer HealthCare AG, GDD-GED-GT Inhalation Toxicology. Bayer MaterialScience AG, Degussa AG (Evonik Degussa GmbH), PERSTORP France SAS, BASF SE, unpublished

Bayer (2010): 1,5-Naphthylene diisocyanate (NDI): 90-day inhalation study with a 1-month recovery period in Wistar rats. Report no. AT06097, study no. T 3080041, date: 2010-11-04. Bayer Schering Pharma AG, Department of General Toxicology - Inhalation. Bayer MaterialScience AG, unpublished

Bayer (2011): MDI22: Local lymph node assay in mice (LLNA/IMDS). Report no. AT06207, study no.: T3081851, date: 2011-02-09. Bayer Schering Pharma AG, Department of Toxicology. Bayer MaterialScience AG, unpublished

Bell S.J.D., Metzger W.J., Welch C.A., and Gilmour M.I. (1996): A role for Th2 T-memory cells in early airway obstruction. Cellular Immunology 170 (2), 185-194. DOI: 10.1006/cimm.1996.0151

Bentley A.M., Maestrelli P., Saetta M., Fabbri L.M., Robinson D.S., Bradley B.L., Jeffery P.K., Durham S.R., and Kay A.B. (1992): Activated T-lymphocytes and eosinophils in the bronchial mucosa in isocyanate-induced asthma. The Journal of Allergy and Clinical Immunology 89 (4), 821-829. DOI: 10.1016/0091-6749(92)90437-7 (last accessed 2016-09-28)

Bernstein D.I., Korbee L., Stauder T., Bernstein J.A., Scinto J., Herd Z.L., and Bernstein I.L. (1993): The low prevalence of occupational asthma and antibody-dependent sensitization to diphenylmethane diisocyanate in a plant engineered for minimal exposure to diisocyanates. Journal of Allergy and Clinical Immunology 92 (3), 387-396. DOI: 10.1016/0091-6749(93)90117-X (last accessed 2016-09-19)

Bernstein I.L., Splansky G.L., Chen S.E., and Vinegar A. (1982): The guinea pig model of diisocyanate sensitization - II. Physiologic studies. Journal of Allergy and Clinical Immunology 70 (5), 393-398. DOI: 10.1016/0091-6749(82)90030-6 (last accessed 2016-09-19)

Bieler G., Thorn D., Huynh C.K., Tomicic C., Steiner U.C., Yawalkar N., and Danuser B. (2011): Acute life-threatening extrinsic allergic alveolitis in a paint controller. Occupational Medicine 61 (6), 440-442. DOI: 10.1093/occmed/kqr057 (last accessed 2016-09-28)

Bio-Dynamics (1984): A closed-patch repeated insult dermal sensitization study in guinea pigs with TDI, MDI, p-TMXDI, IPDI, m-TMXDI, HMDI and m-TMI (modified Buehler method). Report no. 4971-84, date: 1984-12-20. American Cyanamide, unpublished

Bio-Research Laboratories (1984a): Range-finding study with inhaled AC-005 and AC-008 in guinea pigs, unpublished

Bio-Research Laboratories (1984b): Sensitization potential of inhaled AC-005 and AC-008 in guinea pigs. Report no. 81187/88, date: 1984-12-28. Bio-Research Laboratories Ltd. Cytec Industries, unpublished

Blaikie L., Morrow T., Wilson A.P., Hext P., Hartop P.J., Rattray N.J., Woodcock D., Botham P.A., Woodcock D., and Botham P.A. (1995): A two-centre study for the evaluation and validation of an animal model for the assessment of the potential of small molecular weight chemicals to cause respiratory allergy. Toxicology 96 (1), 37-50. DOI: 10.1016/0300-483X(94)03007-O (last accessed 2016-09-19)

Bodner K.M., Burns C.J., Randolph N.M., and Salazar E.J. (2001): A longitudinal study of respiratory health of toluene diisocyanate production workers. Journal of Occupational and Environmental Medicine 43 (10), 890-897. DOI: 10.1097/00043764-200110000-00008 (last accessed 2016-09-19)

Boschetto P., Fabbri L.M., Zocca E., Milani G., Pivirotto F., Dal Vecchio A., Plebani M., and Mapp C.E. (1987): Prednisone inhibits late asthmatic reactions and airway inflammation induced by toluene diisocyanate in sensitized subjects. Journal of Allergy and Clinical Immunology 80 (3 PART 1), 261-267. DOI: 10.1016/0091-6749(87)90028-5 (last accessed 2016-09-28)

Botham P.A., Hext P.M., Rattray N.J., Walsh S.T., and Woodcock D.R. (1988): Sensitisation of guinea pigs by inhalation exposure to low molecular weight chemicals. Toxicology Letters 41 (2), 159-173. DOI: 10.1016/0378-4274(88)90089-6 (last accessed 2016-09-19)

BRC (1981): Dermal sensitisation study of compound number 11583B15 and isophorone diisocyanate. Report no. 81-149, date: 1981-10-23. Biosphere Research Centre. Cytec Industries, unpublished

Bruckner H.C., Avery S.B., Stetson D.M., Dodson V.N., and Ronayne J.J. (1968): Clinical and immunologic appraisal of workers exposed to diisocyanates. Archives of Environmental Health 16 (5), 619-625. DOI: 10.1080/00039896.1968.10665117 (last accessed 2016-09-19)

Bugler J., Clark R.L., and Hill I.D. (1991): The acute and long-term respiratory effects of aromatic di-isocyanates. A five year longitudinal study of polyurethane foam workers. III report 10848. International Isocyanate Institute, Manchester, UK

Burge P.S. (1982): Non-specific bronchial hyper-reactivity in workers exposed to toluene di-isocyanate, diphenyl methane di-isocyanate and colophony. European Journal of Respiratory Diseases Supplement 123, 91-96

Butcher B.T., Jones R.N., O'Neil C., Glindmeyer H.W., Diem J.E., Dharmarajan V., Weill H., and Salvaggio J.E. (1977): Longitudinal study of workers employed in the manufacture of toluene diisocyanate. American Review of Respiratory Disease 116 (3), 411-421. DOI: 10.1164/arrd.1977.116.3.411 (last accessed 2016-09-19)

Butcher B.T., Karr R.M., O'Neil C.E., Wilson M.R., Dharmarajan V., Salvaggio J.E., and Weill H. (1979): Inhalation challenge and pharmacologic studies of toluene diisocyanate (TDI)-sensitive workers. Journal of Allergy and Clinical Immunology 64 (2), 146-152. DOI: 10.1016/0091-6749(79)90049-6 (last accessed 2016-09-19)

Butcher B.T., O'Neil C.E., Reed M.A., and Salvaggio J.E. (1980): Radioallergosorbent testing of toluene diisocyanate-reactive individuals using p-tolyl isocyanate antigen. Journal of Allergy and Clinical Immunology 66 (3), 213-216. DOI: 10.1016/0091-6749(80)90041-X (last accessed 2016-09-28)

Calcagni P., Di Stefano A., Saetta M., Mapp C.E., Fabbri L.M., and Maestrelli P. (1993): Eosinophilia in induced sputum after asthmatic reactions to isocyanates in sensitized subjects. Journal of Allergy and Clinical Immunology 91 (1 PART 2), 221. DOI: 10.1016/0091-6749(93)90360-R (last accessed 2016-09-19)

Carino M., Aliani M., Licitra C., Sarno N., and Ioli F. (1997): Death due to asthma at workplace in a diphenylmethane diisocyanate-sensitized subject. Respiration 64 (1), 111-113. DOI: 10.1159/000196653 (last accessed 2016-09-19)

Cartier A., Grammer L., Malo J.L., Lagier F., Ghezzo H., Harris K., and Patterson R. (1989): Specific serum antibodies against isocyanates: Association with occupational asthma. Journal of Allergy and Clinical Immunology 84 (4 PART 1), 507-514. DOI: 10.1016/0091-6749(89)90364-3 (last accessed 2016-09-19)

Cassidy L.D., Molenaar D.M., Hathaway J.A., Feeley T.M., Cummings B.J., Simpson P., and Li S.H. (2010): Trends in pulmonary function and prevalence of asthma in hexamethylene diisocyanate workers during a 19-year period. Journal of Occupational and Environmental Medicine 52 (10), 988-994. DOI: 10.1097/JOM.0b013e3181f2e086 (last accessed 2016-09-19)

Chang K.C. and Karol M.H. (1984): Diphenylmethane diisocyanate (MDI)-induced asthma: Evaluation of the immunologic responses and application of an animal model of isocyanate sensitivity. Clinical Allergy 14 (4), 329-339. DOI: 10.1111/j.1365-2222.1984.tb02213.x (last accessed 2016-09-19)

Chen S.E. and Bernstein I.L. (1982): The guinea pig model of diisocyanate sensitization. I. Immunologic studies. Journal of Allergy and Clinical Immunology 70 (5), 383-392. DOI: 10.1016/0091-6749(82)90029-X (last accessed 2016-09-19)

Cibulas W., Jr., Brooks S.M., Murlas C.G., Miller M.L., and McKay R.T. (1988): Toluene diisocyanate-induced airway hyperreactivity in guinea pigs depleted of granulocytes. Journal of Applied Physiology 64 (5), 1773-1778. DOI: 10.1152/jappl.1988.64.5.1773

Clark R.L., Bugler J., McDermott M., Hill I.D., Allport D.C., and Chamberlain J.D. (1998): An epidemiology study of lung function changes of toluene diisocyanate foam workers in the United Kingdom. International Archives of Occupational and Environmental Health 71 (3), 169-179. DOI: 10.1007/s004200050267 (last accessed 2016-09-19)

Clark R.L., Bugler J., Paddle G.M., Chamberlain J.D., and Allport D.C. (2003): A 17-year epidemiological study on changes in lung function in toluene diisocyanate foam workers. International Archives of Occupational and Environmental Health 76 (4), 295-301. DOI: 10.1007/s00420-002-0403-8 (last accessed 2016-09-19)

Clemmensen S. (1984): Cross-reaction patterns in guinea pigs sensitized to acrylic monomers. Drug and Chemical Toxicology 7 (6), 527-540. DOI: 10.3109/01480548409042817 (last accessed 2016-09-19)

Cvitanovic S., Zekan L.J., and Marusic M. (1989): Occurrence and specificity of IgE antibodies to isocyanates in occupationally exposed workers. International Archives of Occupational and Environmental Health 61 (7), 483-486. DOI: 10.1007/BF00386483 (last accessed 2016-09-19)

Dahlqvist M., Tornling G., Plato N., and Ulfvarson U. (1995): Effects within the week on forced vital capacity are correlated with long term changes in pulmonary function: Reanalysis of studies on car painters exposed to isocyanate. Occupational and Environmental Medicine 52 (3), 192-195 (last accessed 2016-09-28)

de Jong W.H., Arts J.H.E., de Klerk A., Schijf M.A., Ezendam J., Kuper C.F., and van Loveren H. (2009): Contact and respiratory sensitizers can be identified by cytokine profiles following inhalation exposure. Toxicology 261 (3), 103-111. DOI: 10.1016/j.tox.2009.04.057 (last accessed 2016-09-19)

de Vooght V., Carlier V., Devos F.C., Haenen S., Verbeken E., Nemery B., Hoet P.H., and Vanoirbeek J.A. (2013): B-lymphocytes as key players in chemical-induced asthma. PLoS One 8 (12), e83228. DOI: 10.1371/journal.pone.0083228 (last accessed 2016-09-19)

Dearman R.J., Basketter D.A., and Kimber I. (1992a): Variable effects of chemical allergens on serum IgE concentration in mice: Preliminary evaluation of a novel approach to the identification of respiratory sensitizers. Journal of Applied Toxicology 12 (5), 317-323. DOI: 10.1002/jat.2550120505 (last accessed 2016-09-19)

Dearman R.J., Basketter D.A., and Kimber I. (1996a): Characterization of chemical allergens as a function of divergent cytokine secretion profiles induced in mice. Toxicology and Applied Pharmacology 138 (2), 308-316. DOI: 10.1006/taap.1996.0129 (last accessed 2016-09-19)

Dearman R.J. and Botham P.A. (1990): Inhalation exposure to respiratory sensitising chemicals down-regulates guinea pig IgE and pulmonary responses. International Archives of Allergy and Applied Immunology 92 (4), 425-432. DOI: 10.1159/000235175 (last accessed 2016-09-19)

Dearman R.J. and Kimber I. (2000): Role of CD4+ T helper 2-type cells in cutaneous inflammatory responses induced by fluorescein isothiocyanate. Immunology 101 (4), 442-451. DOI: 10.1046/j.1365-2567.2000.01126.x

Dearman R.J., Moussavi A., Kemeny D.M., and Kimber I. (1996b): Contribution of CD4+ and CD8+ T lymphocyte subsets to the cytokine secretion patterns induced in mice during sensitization to contact and respiratory chemical allergens. Immunology 89 (4), 502-510. DOI: 10.1046/j.1365-2567.1996.d01-778.x (last accessed 2016-09-19)

Dearman R.J., Spence L.M., and Kimber I. (1992b): Characterization of murine immune responses to allergenic diisocyanates. Toxicology and Applied Pharmacology 112 (2), 190-197. DOI: 10.1016/0041-008X(92)90187-W (last accessed 2016-09-19)

Diem J.E., Jones R.N., Hendrick D.J., Glindmeyer H.W., Dharmarajan V., Butcher B.T., Salvaggio J.E., and Weill H. (1982): Five-year longitudinal study of workers employed in a new toluene diisocyanate manufacturing plant. American Review of Respiratory Disease 126 (3), 420-428. DOI: 10.1164/arrd.1982.126.3.420 (last accessed 2016-09-28)

Dietemann-Molard A., Kopferschmitt-Kubler M.C., Meyer P.D., Tomb R., and Pauli G. (1991): Allergic asthma due to domestic use of insulating polyurethane foam. The Lancet 338 (8772), 953. DOI: 10.1016/0140-6736(91)91825-F (last accessed 2016-09-19)

Diller W.F. (2002): Frequency and trends of occupational asthma due to toluene diisocyanate: a critical review. Applied Occupational and Environmental Hygiene 17 (12), 872-877. DOI: 10.1080/10473220290107075 (last accessed 2016-09-19)

Diller W.F. and Herbert E. (1983): Lung function and other health parameters in workers in an isocyanate factory (MDI production). Abstracts on Hygiene and Communicable Diseases 58 (8), 524-525

Donnelly R., Buick J.B., and Macmahon J. (2004): Occupational asthma after exposure to plaster casts containing methylene diphenyl diisocyanate. Occupational Medicine 54 (6), 432-434. DOI: 10.1093/occmed/kqg133 (last accessed 2016-09-19)

Dragos M., Jones M., Malo J.-L., Ghezzo H., and Gautrin D. (2009): Specific antibodies to diisocyanate and work-related respiratory symptoms in apprentice car-painters. Occupational and Environmental Medicine 66 (4), 227-234. DOI: 10.1136/oem.2007.038125 (last accessed 2016-09-19)

DuPont (1971): Production of skin sensitization by inhalation of isocyanates. Report no. 161-71, date: 1971-05-20. DuPont, Haskell Laboratories. DuPont De Nemours & Co., unpublished

DuPont (1974): Immunopathological features of isocyanate compounds. Report no. 249-74, date: 1974-04-16. DuPont, Haskell Laboratories. DuPont De Nemours & Co., unpublished

DuPont (1977): Special primary skin irritation and sensitization tests on guinea pigs. Report no. 95-77, date: 1977-02-25. DuPont, Haskell Laboratories. DuPont De Nemours & Co., unpublished

Duprat P., Gradiski D., and Marignac B. (1976): Irritating and allergising power of two isocyanates. Toluene diisocyanate (TDI) and diphenylmethane diisocyanate (MDI). European Journal of Toxicology 9, 41-53

Ebino K. (1999): The importance of the diluent for airway transport of toluene diisocyanate following intranasal dosing of mice. Inhalation Toxicology 11 (3), 171-185. DOI: 10.1080/089583799197131

Ebino K., Ueda H., Kawakatsu H., Shutoh Y., Kosaka T., Nagayoshi E., Lemus R., and Karol M.H. (2001): Isolated airway exposure to toluene diisocyanate results in skin sensitization. Toxicology Letters 121 (1), 79-85. DOI: 10.1016/S0378-4274(01)00325-3 (last accessed 2016-09-19)

Erban V. (1987): Isocyanatasthma in einer Graugieβerei [Isocyanate astma in a gray iron foundry]. Arbeitsmedizin Sozialmedizin Präventivmedizin 22, 249-253

Erban V. (1988): The bronchial asthma after isocyanate exposure in the foundry workshop. Pracovni Lekartsvi 40 (7), 298-302

Fabbri L.M., Danieli D., Crescioli S., Bevilacqua P., Meli S., Saetta M., and Mapp C.E. (1988): Fatal asthma in a subject sensitized to toluene diisocyanate. American Review of Respiratory Disease 137 (6), 1494-1498. DOI: 10.1164/ajrccm/137.6.1494 (last accessed 2016-09-28)

Farraj A.K., Boykin E., Haykal-Coates N., Gavett S.H., Doerfler D., and Selgrade M. (2007): Th2 cytokines in skin draining lymph nodes and serum IgE do not predict airway hypersensitivity to intranasal isocyanate exposure in mice. Toxicological Sciences 100 (1), 99-108. DOI: 10.1093/toxsci/kfm194 (last accessed 2016-09-19)

Finotto S., Fabbri L.M., Rado V., Mapp C.E., and Maestrelli P. (1991): Increase in numbers of CD8 positive lymphocytes and eosinophils in peripheral blood of subjects with late asthmatic reactions induced by toluene diisocyanate. British Journal of Industrial Medicine 48 (2), 116-121. DOI: 10.1136/oem.48.2.116 (last accessed 2016-09-28)

Friedman S.A. (1982): Fibrosing alveolitis in man following exposure to diphenylmethane diisocyanate: First report. American Review of Respiratory Disease 125 (4)

Fukuyama T., Tajima Y., Ueda H., Hayashi K., Shutoh Y., Harada T., and Kosaka T. (2010): A method for measuring mouse respiratory allergic reaction to low-dose chemical exposure to allergens: An environmental chemical of uncertain allergenicity, a typical contact allergen and a non-sensitizing irritant. Toxicology Letters 195 (1), 35-43. DOI: 10.1016/j.toxlet.2010.02.016 (last accessed 2016-09-19)

Fukuyama T., Ueda H., Hayashi K., Tajima Y., Shuto Y., Saito T.R., Harada T., and Kosaka T. (2008): Use of long term dermal sensitization followed by intratracheal challenge method to identify low-dose chemical-induced respiratory allergic responses in mice. Toxicology Letters 181 (3), 163-170. DOI: 10.1016/j.toxlet.2008.07.017 (last accessed 2016-09-20)

Gad S.C., Dunn B.J., Dobbs D.W., Reilly C., and Walsh R.D. (1986): Development and validation of an alternative dermal sensitization test: The mouse ear swelling test (MEST). Toxicology and Applied Pharmacology 84 (1), 93-114. DOI: 10.1016/0041-008X(86)90419-9 (last accessed 2016-09-20)

Gagnaire F., Ban M., Cour C., Micillino J.C., Bonnet P., and Hettich D. (1997): Role of tachykinins and neutral endopeptidase in toluene diisocyanate-induced bronchial hyperresponsiveness in guinea pigs. Toxicology 116 (1-3), 17-26. DOI: 10.1016/S0300-483X(96)03517-2 (last accessed 2016-09-20)

Gagnaire F., Ban M., Micillino J.C., Lemonnier M., and Bonnet P. (1996): Bronchial responsiveness and inflammation in guinea-pigs exposed to toluene diisocyanate: A study on single and repeated exposure. Toxicology 114 (2), 91-100. DOI: 10.1016/S0300-483X(96)03415-4 (last accessed 2016-09-20)

Gee J.B. and Morgan W.K. (1985): A 10-year follow-up study of a group of workers exposed to isocyanates. Journal of Occupational Medicine 27 (1), 15-18

Gui W., Wisnewski A.V., Neamtiu I., Gurzau E., Sparer J.A., Stowe M.H., Liu J., Slade M.D., Rusu O.A., and Redlich C.A. (2014): Inception cohort study of workers exposed to toluene diisocyanate at a polyurethane foam factory: Initial one-year follow-up. American Journal of Industrial Medicine 57 (11), 1207-1215. DOI: 10.1002/ajim.22385 (last accessed 2016-09-19)

Haag M., Leusink-Muis T., Le Bouquin R., Nijkamp F.P., Lugnier A., Frossard N., Folkerts G., and Pons F. (2002): Increased expression and decreased activity of cytochrome P450 1A1 in a murine model of toluene diisocyanate-induced asthma. Archives of Toxicology 76 (11), 621-627. DOI: 10.1007/s00204-002-0393-z (last accessed 2016-09-20)

Hathaway J.A., DeWilde A., Shepperly D.C., Nguyen L.T., and Johnson J.E. (1999): Evaluation of pulmonary function in workers exposed to hexamethylene diisocyanate. Journal of Occupational and Environmental Medicine 41 (5), 378-383 (last accessed 2016-09-28)

Hazleton (1984): The toxicity and carcinogenicity to rats of toluene diisocyanate vapour administered by inhalation for a period of 113 weeks. Addendum Report Volume. III project A-A-1, III report 10233, date: 1984-03-01. Hazleton Laboratories Europe Ltd. International Isocyanate Institute, unpublished

Hazleton (1986): The toxicity and carcinogenicity of toluene diisocyanate vapour when administered to mice over a period of approximately two years. Volumes I and II. III project A-A-1, III Report 10382, date: 1986-01-03. Hazleton Laboratories Europe Ltd. International Isocyanate Institute, unpublished

Hesbert A., Ban M., Bonnet P., Simon P., Bottin M.C., Lemonnier M., and de Ceaurriz J. (1991): Interdependence of polymorphonuclear neutrophils and macrophages stained for N-acetyl-beta-glucosaminidase in lavage effluents from toluene diisocyanate-exposed rat lungs. Toxicology Letters 56 (1-2), 53-59. DOI: 10.1016/0378-4274(91)90089-O (last accessed 2016-09-20)

Hilton J., Dearman R.J., Basketter D.A., and Kimber I. (1995): Identification of chemical respiratory allergens: Dose-response relationships in the mouse IgE test. Toxicology Mechanisms and Methods 5 (1), 51-60. DOI: doi: 10.3109/15376519509066117 (last accessed 2016-09-20)

Hilton J., Dearman R.J., Debicki R.J., Ramdin L.S.P., and Kimber I. (1994): Interleukin 6 production in vitro: An alternative read-out for the local lymph node assay. Toxicology In Vitro 8 (4), 711-713. DOI: 10.1016/0887-2333(94)90049-3 (last accessed 2016-09-20)

Holness D.L., Broder I., Corey P.N., Booth N., Mozzon D., Nazar M.A., and Guirguis S. (1984): Respiratory variables and exposure-effect relationships in isocyanate-exposed workers. Journal of Occupational Medicine 26 (6), 449-455

Hoymann H.G., Buschmann J., and Heinrich U. (1995): Untersuchungen zur chronischen Toxizität/Kanzerogenität von 4,4’-Methylendiphenyl-Diisocyanat (MDI) [Studies on the chronic toxicity/carcinogenicity of 4,4'-methylenediphenyl-diisocyanate (MDI)]. Forschungsbericht 116 06 084, date: 1995-09-01. Fraunhofer-Institut für Toxikologie und Aerosolforschung. Umweltbundesamt (UBA)

Huang J., Wang X.P., Chen B.M., Ueda A., Aoyama K., and Matsushita T. (1991): Immunological effects of toluene diisocyanate exposure on painters. Archives of Environmental Contamination and Toxicology 21 (4), 607-611. DOI: 10.1007/BF01183884 (last accessed 2016-09-20)

Huang J., Wang X.P., Chen B.M., Zhou X.J., and Matsushita T. (1993): Dose-response relationships for chemical sensitization from TDI and DNCB. Bulletin of Environmental Contamination and Toxicology 51 (5), 732-739. DOI: 10.1007/BF00201652 (last accessed 2016-09-20)

Huffman L.J., Judy D.J., Frazer D., Shapiro R.E., Castranova V., Billie M., and Dedhia H.V. (1997): Inhalation of toluene diisocyanate is associated with increased production of nitric oxide by rat bronchoalveolar lavage cells. Toxicology and Applied Pharmacology 145 (1), 61-67. DOI: 10.1006/taap.1997.8162 (last accessed 2016-09-20)

Huntingdon (1980): Screening test for delayed contact hypersensitivity with 1,3-bis(isocyanatomethyl)benzene in the albino guinea-pig. Report no. 80308D/TDA/77, date: 1980-04-24. Huntingdon Research Centre. Takeda Chemical Industries Ltd., unpublished

Huntingdon (1997): Takenate 500 skin sensitisation in the guinea-pig. Report no. TDA 217/971230, date: 1997-06-02. Huntingdon Life Sciences Ltd. Takeda Chemical Industries Ltd., unpublished

IBR (1977): Prüfung von "IPDI-T 1890" im epicutanen Sensibilisierungstest an der Meerschweinchenhaut (25 %ig in Sojabohnenöl) [Study of "IPDI-T 1890" in the epicutaneous sesnitisation test in guinea-pig skin (25% in soy bean oil)]. Report no. 2-5-379-77, date: 1977-11-30. International Bio-Research. Evonik Degussa GmbH, unpublished

IBR (1983a): 3-Isocyanatomethyl-3.5.5-trimethylcyclohexylisocyanat - Prüfung auf sensibilisierende Eigenschaften am Meerschweinchen nach B. Magnusson und A.M. Kligman (gemäß OECD Richtlinien) [3-Isocyanatomethyl-3.5.5-trimethylcyclohexylisocyanate - test for sensitising properties in the guinea-pig according to B. Magnusson and A.M. Kligman (following OECD guidelines)]. Report no. 2-5-120-83, date: 1983-07-11. IBR Forschungs GmbH. Evonik Degussa GmbH, unpublished

IBR (1983b): Polyisocyanat IPDI-T 1890S (monomeres IPDI: < 0,5 Gew. %) - Prüfung auf sensibilisierende Eigenschaften am Meerschweinchen nach B. Magnusson und A.M. Kligman (gemäß OECD Richtlinien) [Polyisocyanate IPDI-T 1890S (monomeric IPDI: < 0.5% by wt.) - test for sensitising properties in the guinea-pig according to B. Magnusson and A.M. Kligman (following OECD guidelines)]. Report no. 2-5-348-83, date: 1983-09-30. IBR Forschungs GmbH. Evonik Degussa GmbH, unpublished

Innocenti A., Cirla A.M., Pisati G., and Mariano A. (1988): Cross-reaction between aromatic isocyanates (TDI and MDI): A specific bronchial provocation test study. Clinical Allergy 18 (4), 323-329

Innocenti A. and Paggiaro P.L. (1983): A case of occupational asthma due to diphenylmethane diisocyanate (MDI). Medicina del Lavoro 74 (5), 391-393

Jang A.S., Choi I.S., Koh Y.I., Moon J.D., and Lee K.J. (2000): Increase in airway hyperresponsiveness among workers exposed to methylene diphenyldiisocyanate compared to workers exposed to toluene diisocyanate at a petrochemical plant in Korea. American Journal of Industrial Medicine 37 (6), 663-667. DOI: 10.1002/(SICI)1097-0274(200006)37:6<663::AID-AJIM11>3.0.CO;2-H (last accessed 2016-09-20)

Jin R. and Karol M.H. (1988): Diisocyanate antigens that detect specific antibodies in exposed workers and guinea pigs. Chemical Research in Toxicology 1 (5), 288-293. DOI: 10.1021/tx00005a006 (last accessed 2016-09-20)

Johnson A., Moira C.Y., MacLean L., Atkins E., Dybuncio A., Cheng F., and Enarson D. (1985): Respiratory abnormalities among workers in an iron and steel foundry. British Journal of Industrial Medicine 42 (2), 94-100 (last accessed 2016-09-28)

Jones R.N., Rando R.J., Glindmeyer H.W., Foster T.A., Hughes J.M., O'Neil C.E., and Weill H. (1992): Abnormal lung function in polyurethane foam producers: Weak relationship to toluene diisocyanate exposures. American Review of Respiratory Disease 146 (4), 871-877. DOI: 10.1164/ajrccm/146.4.871 (last accessed 2016-09-19)

Kakooei H., Shahtaheri S.J., and Karbasi H.A. (2006): Evaluation of workers' exposure to methylene diphenyl diisocyanate (MDI) in an automobile manufacturing company, Iran. International Journal of Occupational Safety and Ergonomics 12 (4), 443-449. DOI: 10.1080/10803548.2006.11076701 (last accessed 2016-09-20)

Kalubi B., Takeda N., Irifune M., Ogino S., Abe Y., Hong S.L., Yamano M., Matsunaga T., and Tohyama M. (1992): Nasal-mucosa sensitization with toluene diisocyanate (TDI) increases preprotachykinin A (PPTA) and preproCGRP mRNAs in guinea pig trigeminal ganglion neurons. Brain Research 576 (2), 287-296. DOI: 10.1016/0006-8993(92)90692-3 (last accessed 2016-09-20)

Kanerva L., Grenquist-Nordén B., and Piirilä P. (1999): Occupational IgE-mediated contact urticaria from diphenylmethane-4,4'-diisocyanate (MDI). Contact Dermatitis 41 (1), 50-51. DOI: 10.1111/j.1600-0536.1999.tb06215.x (last accessed 2016-09-20)

Karol M.H. (1983): Concentration-dependent immunologic response to toluene diisocyanate (TDI) following inhalation exposure. Toxicology and Applied Pharmacology 68 (2), 229-241. DOI: 10.1016/0041-008X(83)90007-8 (last accessed 2016-09-20)

Karol M.H., Hauth B.A., Riley E.J., and Magreni C.M. (1981): Dermal contact with toluene diisocyanate (TDI) produces respiratory tract hypersensitivity in guinea pigs. Toxicology and Applied Pharmacology 58 (2), 221-230. DOI: 10.1016/0041-008X(81)90426-9 (last accessed 2016-09-19)

Karol M.H. and Kramarik J.A. (1996): Phenyl isocyanate is a potent chemical sensitizer. Toxicology Letters 89 (2), 139-146. DOI: 10.1016/S0378-4274(96)03798-8 (last accessed 2016-09-20)

Karol M.H. and Magreni C. (1982): Extensive skin sensitization with minimal antibody production in guinea pigs as a result of exposure to dicyclohexylmethane-4,4′-diisocyanate. Toxicology and Applied Pharmacology 65 (2), 291-301. DOI: 10.1016/0041-008X(82)90012-6 (last accessed 2016-09-20)

Kilgour J.D., Rattray N.J., Foster J., Soames A., and Hext P.M. (2002): Pulmonary responses and recovery following single and repeated inhalation exposure of rats to polymeric methylene diphenyl diisocyanate aerosols. Journal of Applied Toxicology 22 (6), 371-385. DOI: 10.1002/jat.872 (last accessed 2016-09-20)

Kim H., Kim Y.D., and Choi J. (1997): Seroimmunological characteristics of Korean workers exposed to toluene diisocyanate. Environmental Research 75 (1), 1-6. DOI: 10.1006/enrs.1997.3763 (last accessed 2016-09-28)

Koschier F.J., Burden E.J., Brunkhorst C.S., and Friedman M.A. (1983): Concentration-dependent elicitation of dermal sensitization in guinea pigs treated with 2,4-toluene diisocyanate. Toxicology and Applied Pharmacology 67 (3), 401-407. DOI: 10.1016/0041-008X(83)90324-1 (last accessed 2016-09-20)

Kouadio K., Zheng K.C., Tuekpe M.K.-N., Todoriki H., and Ariizumi M. (2005): Airway inflammatory and immunological events in a rat model exposed to toluene diisocyanate. Food and Chemical Toxicology 43 (8), 1281-1288. DOI: 10.1016/j.fct.2005.03.005 (last accessed 2016-09-20)

Laitinen J., Muittari A., and Sovijärvi A. (1984): Isosyanaatit astman ja alveoliitin aiheuttajina [Diisocyanate-induced asthma and alveolitis]. Duodecim 100 (4), 220-225. http://www.terveysportti.fi/d-htm/articles/1984_4_220-225.pdf (last accessed 2016-09-20)

Lee H.S. and Phoon W.H. (1992): Diurnal variation in peak expiratory flow rate among workers exposed to toluene diisocyanate in the polyurethane foam manufacturing industry. British Journal of Industrial Medicine 49 (6), 423-427. http://oem.bmj.com/content/49/6/423.abst (last accessed 2016-09-28)

Lee Y.C., Kwak Y.G., and Song C.H. (2002): Contribution of vascular endothelial growth factor to airway hyperresponsiveness and inflammation in a murine model of toluene diisocyanate-induced asthma. Journal of Immunology 168 (7), 3595-3600. DOI: 10.4049/jimmunol.168.7.3595 (last accessed 2016-09-20)

Lee Y.M., Kim H.A., Park H.S., Lee S.K., and Nahm D.H. (2003): Exposure to toluene diisocyanate (TDI) induces IL-8 production from bronchial epithelial cells: Effect of preinflammatory cytokines. Journal of Korean Medical Science 18 (6), 809-812. DOI: 10.3346/jkms.2003.18.6.809 (last accessed 2016-09-20)

Lemière C., Cartier A., Dolovich J., Chan-Yeung M., Grammer L., Ghezzo H., L'Archeveque J., and Malo J.L. (1996): Outcome of specific bronchial responsiveness to occupational agents after removal from exposure. American Journal of Respiratory and Critical Care Medicine 154 (2), 329-333. DOI: 10.1164/ajrccm.154.2.8756802 (last accessed 2016-09-20)

Lenaerts-Langanke H. (1992): Occupational lung diseases in coal miners: Isocyanate-induced respiratory problems, 929-933. Eighth International Conference on Occupational Lung Diseases, Prague, Czechoslovakia, Czech Medical Society,

Liang J., Zhao H., Yao L., Tang H., Dong H., Wu Y., Liu L., Zou F., and Cai S. (2015): Phosphatidylinositol 3-kinases pathway mediates lung caspase-1 activation and high mobility group box 1 production in a toluene-diisocyanate induced murine asthma model. Toxicology Letters 236 (1), 25-33. DOI: 10.1016/j.toxlet.2015.04.011 (last accessed 2016-09-20)

Lim R.H., Arredouani M.S., Fedulov A., Kobzik L., and Hubeau C. (2007): Maternal allergic contact dermatitis causes increased asthma risk in offspring. Respiratory Research 8, 56. DOI: 10.1186/1465-9921-8-56 (last accessed 2016-09-20)

Lindberg H.K., Korpi A., Santonen T., Säkkinen K., Järvelä M., Tornaeus J., Ahonen N., Järventaus H., Pasanen A.L., Rosenberg C., and Norppa H. (2011): Micronuclei, hemoglobin adducts and respiratory tract irritation in mice after inhalation of toluene diisocyanate (TDI) and 4,4'-methylenediphenyl diisocyanate (MDI). Mutation Research/Genetic Toxicology and Environmental Mutagenesis 723 (1), 1-10. DOI: 10.1016/j.mrgentox.2011.03.009 (last accessed 2016-09-20)

Littorin M., Axmon A., Broberg K., Sennbro C.J., and Tinnerberg H. (2007): Eye and airway symptoms in low occupational exposure to toluene diisocyanate. Scandinavian Journal Of Work Environment & Health 33 (4), 280-285. http://www.jstor.org/stable/40967655 (last accessed 2016-09-28)

Lob M. and Boillat M.A. (1981): Asthme retarde au diphenylmethane-diisocyanate (MDI). Importance de l'anamnese professionnelle. Utilité et danger des tests inhalatoires. A propos de cinq cas [Delayed asthma on diphenylmethane diisocyanate (MDI). The importance of a professional history. The utility and danger of the inhalation tests. Apropos five cases]. Schweizerische Medizinische Wochenschrift 111 (5), 150-154

Ma-Hock L., Gamer A.O., Deckardt K., Leibold E., and van Ravenzwaay B. (2007): Determination of pulmonary irritant threshold concentrations of hexamethylene-1,6-diisocyanate (HDI) prepolymers by bronchoalveolar lavage in acute rat inhalation studies according to TRGS 430. Food and Chemical Toxicology 45 (2), 237-243. DOI: 10.1016/j.fct.2006.08.016 (last accessed 2016-09-20)

Maestrelli P., Calcagni P.G., Saetta M., Di S.A., Hosselet J.J., Santonastaso A., Fabbri L.M., and Mapp C.E. (1994a): Sputum eosinophilia after asthmatic responses induced by isocyanates in sensitized subjects. Clinical and Experimental Allergy 24 (1), 29-34. DOI: 10.1111/j.1365-2222.1994.tb00913.x (last accessed 2016-09-20)

Maestrelli P., Del Prete G.F., De Carli M., D'Elios M.M., Saetta M., Di Stefano A., Mapp C.E., Romagnani S., and Fabbri L.M. (1994b): CD8 T-cell clones producing interleukin-5 and interferon-gamma in bronchial mucosa of patients with asthma induced by toluene diisocyanate. Scandinavian Journal Of Work Environment & Health 20 (5), 376-381. http://www.jstor.org/stable/40966279 (last accessed 2016-09-20)

Malo J.L., Ghezzo H., and Elie R. (1999): Occupational asthma caused by isocyanates. American Journal of Respiratory and Critical Care Medicine 159 (6), 1879-1883. DOI: 10.1164/ajrccm.159.6.9806159 (last accessed 2016-09-28)

Malo J.L., L'Archevêque J., and Cartier A. (1989): Significant changes in nonspecific bronchial responsiveness after isolated immediate bronchospecific reactions caused by isocyanates but not after a late reaction caused by plicatic acid. Journal of Allergy and Clinical Immunology 83 (1), 159-165. DOI: 10.1016/0091-6749(89)90491-0 (last accessed 2016-09-20)

Malo J.L., Ouimet G., Cartier A., Levitz D., and Zeiss C.R. (1983): Combined alveolitis and asthma due to hexamethylene diisocyanate (HDI), with demonstration of crossed respiratory and immunologic reactivities to diphenylmethane diisocyanate (MDI). Journal of Allergy and Clinical Immunology 72 (4), 413-419. DOI: 10.1016/0091-6749(83)90508-0 (last accessed 2016-09-20)

Mapp C.E., Dal Vecchio L., Boschetto P., and Fabbri L.M. (1985): Combined asthma and alveolitis due to diphenylmethane diisocyanate (MDI) with demonstration of no crossed respiratory reactivity to toluene diisocyanate (TDI). Annals of Allergy 54 (5), 424-429

Mapp C.E., Silva J.R.L.E., Lucchini R.E., Chitano P., Rado V., Saetta M., Pretolani M., Karol M.H., Maestrelli P., and Fabbri L.M. (1996): Inflammatory events in the blood and airways of guinea pigs immunized to toluene diisocyanate. American Journal of Respiratory and Critical Care Medicine 154 (1), 201-208. DOI: 10.1164/ajrccm.154.1.8680681 (last accessed 2016-09-20)

Marcali K. (1957): Microdetermination of toluenediisocyanates in atmosphere. Analytical Chemistry 29 (4), 552-558. DOI: 10.1021/ac50162a039 (last accessed 2016-10-07)

Matheson J.M., Johnson V.J., and Luster M.I. (2005a): Immune mediators in a murine model for occupational asthma: Studies with toluene diisocyanate. Toxicological Sciences 84 (1), 99-109. DOI: 10.1093/toxsci/kfi051 (last accessed 2016-09-20)

Matheson J.M., Johnson V.J., Vallyathan V., and Luster M.I. (2005b): Exposure and immunological determinants in a murine model for toluene diisocyanate (TDI) asthma. Toxicological Sciences 84 (1), 88-98. DOI: 10.1093/toxsci/kfi050 (last accessed 2016-09-19)

Matheson J.M., Lange R.W., Lemus R., Karol M.H., and Luster M.I. (2001): Importance of inflammatory and immune components in a mouse model of airway reactivity to toluene diisocyanate (TDI). Clinical and

Experimental Allergy 31 (7), 1067-1076. DOI: 10.1046/j.1365-2222.2001.01125.x (last accessed 2016-09-20)

Matheson J.M., Lemus R., Lange R.W., Karol M.H., and Luster M.I. (2002): Role of tumor necrosis factor in toluene diisocyanate asthma. American Journal of Respiratory Cell and Molecular Biology 27 (4), 396-405. DOI: 10.1165/rcmb.4614 (last accessed 2016-09-20)

Matsushima H., Takayanagi N., Tokunaga D., Maeno Y., Sato N., Kurashima K., Ubukata M., Yanagisawa T., Sugita Y., Kawabata Y., and Kanazawa M. (2003): [A case of combined hypersensitivity pneumonitis and bronchial asthma due to isocyanate (MDI)]. Nihon Kokyuki Gakkai Zasshi 41 (10), 760-765. http://www.jrs.or.jp/quicklink/journal/nopass_pdf/041100760j.pdf (last accessed 2016-09-20)

Meredith S.K., Bugler J., and Clark R.L. (2000): Isocyanate exposure and occupational asthma: A case-referent study. Occupational and Environmental Medicine 57 (12), 830-836. DOI: 10.1136/oem.57.12.830 (last accessed 2016-09-20)

Mobay (1988): 90-Day inhalation toxicity study with 1,6-hexamethylene diisocyanate (HDI) in rats. Report no. 81-141-01, date: 1988-12-28. Bayer, unpublished

Mobay (1989): Chronic inhalation toxicity and oncogenicity study with 1,6 hexamethylene diisocyanate (HDI) in rats. Report no. 1157, company study no. 83-241-01, date: 1989-12-05. Bayer, unpublished

Moller D.R., McKay R.T., Bernstein I.L., and Brooks S.M. (1986): Persistent airways disease caused by toluene diisocyanate. American Review of Respiratory Disease 134 (1), 175-176. DOI: 10.1164/arrd.1986.134.1.175 (last accessed 2016-09-28)

Morimatsu Y., Akiyoshi H., Tajiri M., Hirai R., and Aizawa H. (2004): [A case of hypersensitivity pneumonitis showing acute respiratory distress syndrome due to exposure to dust containing diphenylmethane diisocyanate]. Nihon Kokyuki Gakkai Zasshi 42 (6), 502-508. http://www.jrs.or.jp/quicklink/journal/nopass_pdf/042060502j.pdf (last accessed 2016-09-20)

Musk A.W., Peters J.M., and Berstein L. (1985): Absence of respiratory effects in subjects exposed to low concentrations to TDI and MDI: A reevaluation. Journal of Occupational Medicine 27 (12), 917-920. http://journals.lww.com/joem/Abstract/1985/12000/Absence_of_Respiratory_Effects_in_Subjects_Exposed.16.aspxeid=2-s2.0-0022384692&partnerID=40&md5=8c3cab998c523fc6012ce4fb66fb0445 (last accessed 2016-09-20)

Musk A.W., Peters J.M., DiBerardinis L., and Murphy R.L.H. (1982): Absence of respiratory effects in subjects exposed to low concentrations of TDI and MDI. Journal of Occupational Medicine 24 (10), 746-750. http://journals.lww.com/joem/Abstract/1982/10000/Absence_of_Respiratory_Effects_in_Subjects_Exposed.11.aspx (last accessed 2016-09-20)

Nabe T., Yamauchi K., Shinjo Y., Niwa T., Imoto K., Koda A., and Kohno S. (2005): Delayed-type asthmatic response induced by repeated intratracheal exposure to toluene-2,4-diisocyanate in guinea pigs. International Archives of Allergy and Applied Immunology 137 (2), 115-124. DOI: 10.1159/000085466 (last accessed 2016-09-20)

Nayak A.P., Hettick J.M., Siegel P.D., Anderson S.E., Long C.M., Green B.J., and Beezhold D.H. (2014): Toluene diisocyanate (TDI) disposition and co-localization of immune cells in hair follicles. Toxicological Sciences 140 (2), 327-337. DOI: 10.1093/toxsci/kfu079 (last accessed 2016-09-20)

Nemery B. and Lenaerts L. (1993): Exposure to methylene diphenyl diisocyanate in coal mines. The Lancet 341 (8840), 318. DOI: 10.1016/0140-6736(93)92682-J (last accessed 2016-09-20)

Niewenhuis R., Scheel L., Stemmer K., and Killens R. (1965): Toxicity of chronic low level exposures to toluene diisocyanate in animals. American Industrial Hygiene Association Journal 26 (2), 143-149. DOI: 10.1080/00028896509342714 (last accessed 2016-09-20)

Niimi A., Amitani R., Yamada K., Tanaka K.I., and Kuze F. (1996): Late respiratory response and associated eosinophilic inflammation induced by repeated exposure to toluene diisocyanate in guinea pigs. Journal of Allergy and Clinical Immunology 97 (6), 1308-1319. DOI: 10.1016/S0091-6749(96)70200-2 (last accessed 2016-09-20)

NOTOX (1996): Assessment of contact hypersensitivity to polyisocyanate resin 95222660 in the albino guinea pig (maximization test). Report no. 142649, date: 1996-02-02. NOTOX B.V. Bayer MaterialScience AG, unpublished

NOTOX (2004): Assessment of contact hypersensitivity to Vestanat T 1890 SV in the albino guinea pig (maximisation-test). Report no. 397496, date: 2004-04-05. NOTOX B.V. Evonik Degussa GmbH, unpublished

Nozawa S., Sato T., Sasagawa M., Suzuki E., Kioi S., and Arakawa M. (1989): [A case of hypersensitivity pneumonitis due to isocyanate (TDI)]. Nihon Kyobu Shikkan Gakkai Zasshi 27 (11), 1335-1341. DOI: 10.11389/jjrs1963.27.1335 (last accessed 2016-09-20)

O'Brien I.M., Harries M.G., Burge P.S., and Pepys J. (1979): Toluene di-isocyanates-induced asthma. I. Reactions to TDI, MDI, HDI and histamine. Clinical Allergy 9 (1), 1-6. DOI: 10.1111/j.1365-2222.1979.tb01516.x (last accessed 2016-09-28)

Olsen G.W., Shellenberger R., Bodner K.M., Flores G.H., Emmitte J.A., Bond G.G., and Saunders J.H. (1989): An epidemiologic investigation of forced expiratory volume at 1 second and respiratory symptoms among employees of a toluene diisocyanate production plant. Journal of Occupational Medicine 31 (8), 664-667 (last accessed 2016-09-20)

Omae K. (1984): Two-year observation of pulmonary function in workers exposed to low concentrations of toluene diisocyanate. International Archives of Occupational and Environmental Health 55 (1), 1-12. DOI: 10.1007/BF00378062 (last accessed 2016-09-20)

Omae K., Nakadate T., Higashi T., Nakaza M., Aizawa Y., and Sakurai H. (1992): Four-year follow-up of effects of toluene diisocyanate exposure on the respiratory system in polyurethane foam manufacturing workers. I. Study design and results of the first cross-sectional observation. International Archives of Occupational and Environmental Health 63 (8), 559-564. DOI: 10.1007/BF00386346 (last accessed 2016-09-20)

Ott M.G. (2002): Occupational asthma, lung function decrement, and toluene diisocyanate (TDI) exposure: A critical review of exposure-response relationships. Applied Occupational and Environmental Hygiene 17 (12), 891-901. DOI: 10.1080/10473220290107093 (last accessed 2016-09-19)

Ott M.G., Diller W.F., and Jolly A.T. (2003): Respiratory effects of toluene diisocyanate in the workplace: A discussion of exposure-response relationships. Critical Reviews in Toxicology 33 (1), 1-59. DOI: 10.1080/713611031 (last accessed 2016-09-19)

Ott M.G., Klees J.E., and Poche S.L. (2000): Respiratory health surveillance in a toluene di-isocyanate production unit, 1967-97: Clinical observations and lung function analyses. Occupational and Environmental Medicine 57 (1), 43-52. DOI: 10.1136/oem.57.1.43 (last accessed 2016-09-19)

Paggiaro P., Bacci E., Paoletti P., Bernard P., Dente F.L., Marchetti G., Talini D., Menconi G.F., and Giuntini C. (1990): Bronchoalveolar lavage and morphology of the airways after cessation of exposure in asthmatic subjects sensitized to toluene diisocyanate. CHEST 98 (3), 536-542. DOI: 10.1378/chest.98.3.536 (last accessed 2016-09-20)

Park H.S., Park J.N., Kim J.W., and Kim S.K. (1992): Clinical and immunological evaluation of isocyanate-exposed workers. Journal of Korean Medical Science 7 (2), 122-127. DOI: 10.3346/jkms.1992.7.2.122 (last accessed 2016-09-28)

Parker D.L., Waller K., Himrich B., Martinez A., and Martin F. (1991): A cross-sectional study of pulmonary function in autobody repair workers. American Journal of Public Health 81 (6), 768-771. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1405165/ (last accessed 2016-09-28)

Patterson R., Harris K.E., Pruzansky J.J., and Zeiss C.R. (1982): An animal model of occupational immunologic asthma due to diphenylmethane diisocyanate, with multiple systemic immunologic responses. Journal of Laboratory and Clinical Medicine 99 (5), 615-623. http://www.translationalres.com/article/0022-2143%2882%2990308-0/fulltext (last accessed 2016-09-20)

Patterson R., Nugent K.M., Harris K.E., and Eberle M.E. (1990): Immunologic hemorrhagic pneumonia caused by isocyanates. American Review of Respiratory Disease 141 (1), 226-230. DOI: 10.1164/ajrccm/141.1.226 (last accessed 2016-09-28)

Pauluhn J. (1994): Assessment of chemicals for their potential to induce respiratory allergy in guinea pigs: A comparison of different routes of induction and confounding effects due to pulmonary hyperreactivity. Toxicology in Vitro 8 (5), 981-985. DOI: 10.1016/0887-2333(94)90231-3 (last accessed 2016-09-20)

Pauluhn J. (2000a): Acute inhalation toxicity of polymeric diphenyl-methane 4,4'-diisocyanate in rats: Time course of changes in bronchoalveolar lavage. Archives of Toxicology 74 (4-5), 257-269. DOI: 10.1007/s002040000114 (last accessed 2016-09-20)

Pauluhn J. (2000b): Inhalation toxicity of 1,6-hexamethylene diisocyanate homopolymer (HDI-IC) aerosol: Results of single inhalation exposure studies. Toxicological Sciences 58 (1), 173-181. DOI: 10.1093/toxsci/58.1.173 (last accessed 2016-09-20)

Pauluhn J. (2002a): Critical analysis of biomonitoring endpoints for measuring exposure to polymeric diphenyl-methane-4,4'-diisocyanate (MDI) in rats: A comparison of markers of exposure and markers of effect. Archives of Toxicology 76 (1), 13-22. DOI: 10.1007/s00204-001-0301-y (last accessed 2016-09-20)

Pauluhn J. (2002b): Short-term inhalation toxicity of polyisocyanate aerosols in rats: Comparative assessment of irritant-threshold concentrations by bronchoalveolar lavage. Inhalation Toxicology 14 (3), 287-301. DOI: 10.1080/08958370252809068 (last accessed 2016-09-20)

Pauluhn J. (2005): Brown Norway rat asthma model of diphenylmethane 4,4'-diisocyanate. Inhalation Toxicology 17 (13), 729-739. DOI: 10.1080/08958370500224631 (last accessed 2016-09-20)

Pauluhn J. (2008a): Brown Norway rat asthma model of diphenylmethane-4,4'-diisocyanate (MDI): Analysis of the elicitation dose-response relationship. Toxicological Sciences 104 (2), 320-331. DOI: 10.1093/toxsci/kfn098 (last accessed 2016-09-20)

Pauluhn J. (2008b): Brown Norway rat asthma model of diphenylmethane-4,4'-diisocyanate (MDI): Impact of vehicle for topical induction. Regulatory Toxicology and Pharmacology 50 (1), 144-154. DOI: 10.1016/j.yrtph.2007.09.003 (last accessed 2016-09-20)

Pauluhn J. (2014): Development of a respiratory sensitization/elicitation protocol of toluene diisocyanate (TDI) in Brown Norway rats to derive an elicitation-based occupational exposure level. Toxicology 319, 10-22. DOI: 10.1016/j.tox.2014.02.006 (last accessed 2016-09-20)

Pauluhn J. (2015): Analysis of the interrelationship of the pulmonary irritation and elicitation thresholds in rats sensitized with 1,6-hexamethylene diisocyanate (HDI). Inhalation Toxicology 27 (4), 191-206. DOI: 10.3109/08958378.2015.1026619 (last accessed 2016-09-20)

Pauluhn J. and Eben A. (1991): Validation of a non-invasive technique to assess immediate or delayed onset of airway hypersensitivity in guinea-pigs. Journal of Applied Toxicology 11 (6), 423-431. DOI: 10.1002/jat.2550110608 (last accessed 2016)

Pauluhn J., Emura M., Mohr U., Popp A., and Rosenbruch M. (1999): Two-week inhalation toxicity of polymeric diphenylmethane-4,4'-diisocyanate (PMDI) in rats: Analysis of biochemical and morphological markers of early pulmonary response. Inhalation Toxicology 11 (12), 1143-1163. DOI: 10.1080/089583799196637 (last accessed 2016-09-20)

Pauluhn J. and Mohr U. (1998): Assessment of respiratory hypersensitivity in guinea pigs sensitized to toluene diisocyanate: A comparison of sensitization protocols. Inhalation Toxicology 10 (2), 131-154. DOI: 10.1080/089583798197790 (last accessed 2016-09-20)

Pauluhn J. and Mohr U. (2001): Inhalation toxicity of 1,6-hexamethylene diisocyanate homopolymers (HDI-IC and HDI-BT): Results of subacute and subchronic repeated inhalation exposure studies. Inhalation Toxicology 13 (6), 513-532. DOI: 10.1080/08958370118600 (last accessed 2016-09-20)

Pauluhn J. and Poole A. (2011): Brown Norway rat asthma model of diphenylmethane-4,4'-diisocyanate (MDI): Determination of the elicitation threshold concentration of after inhalation sensitization. Toxicology 281 (1-3), 15-24. DOI: 10.1016/j.tox.2011.01.005 (last accessed 2016-09-20)

Pauluhn J., Thiel A., Emura M., and Mohr U. (2000): Respiratory sensitization to diphenyl-methane-4,4'-diisocyanate (MDI) in guinea pigs: Impact of particle size on induction and elicitation of response. Toxicological Sciences 56 (1), 105-113. DOI: 10.1093/toxsci/56.1.105 (last accessed 20160-09-20)

Pauluhn J. and Vohr H.-W. (2006): Partial C-fiber ablation modulates diphenylmethane-4,4′-diisocyanate (MDI)-induced respiratory allergy in Brown Norway rats. Toxicology 228 (2–3), 188-199. DOI: 10.1016/j.tox.2006.08.031 (last accessed 2016-09-20)

Pauluhn J., Woolhiser M.R., and Bloemen L. (2005): Repeated inhalation challenge with diphenylmethane-4,4'-diisocyanate in Brown Norway rats leads to a time-related increase of neutrophils in bronchoalveolar lavage after topical induction. Inhalation Toxicology 17 (2), 67-78. DOI: 10.1080/08958370590898434 (last accessed 2016-09-20)

Perfetti L., Bram, B., Ferrari M., and Moscato G. (2003): Occupational asthma (OA) with sensitization to diphenylmethane diisocyanate (MDI) presenting at the onset like a reactive airways dysfunction syndrome (RADS). American Journal of Industrial Medicine 44 (3), 325-328. DOI: 10.1002/ajim.10262 (last accessed 2016-09-20)

Perrin B., Cartier A., Ghezzo H., Grammer L., Harris K., Chan H., Chan-Yeung M., and Malo J.L. (1991): Reassessment of the temporal patterns of bronchial obstruction after exposure to occupational sensitizing agents. Journal of Allergy and Clinical Immunology 87 (3), 630-639. DOI: 10.1016/0091-6749(91)90381-W (last accessed 2016-09-28)

Pfaffl M.W. (2001): A new mathematical model for relative quantification in real-time RT-PCR. Nucleic acids research 29 (9), e45

Pham Q.T., Cavelier C., Mereau P., Mur J.M., and Cicolella A. (1978): Isocyanates and respiratory function: A study of workers producing polyurethane foam moulding. Annals of Occupational Hygiene 21 (2), 121-129. DOI: 10.1093/annhyg/21.2.121 (last accessed 2016-09-20)

Pham Q.T., Teculescu D., Meyer-Bisch C., and Mur J.M. (1988): Effects of chronic exposure to diisocyanates. Bulletin Européen de Physiopathologie Respiratoire 23, 561-564

Piirilä P.L., Meuronen A., Majuri M.L., Luukkonen R., Mäntylä T., Wolff H.J., Nordman H., Alenius H., and Laitinen A. (2008): Inflammation and functional outcome in diisocyanate-induced asthma after cessation of exposure. Allergy: European Journal of Allergy and Clinical Immunology 63 (5), 583-591. DOI: 10.1111/j.1398-9995.2007.01606.x (last accessed 2016-09-20)

Pisati G., Baruffini A., Bernabeo F., Cerri S., and Mangili A. (2007): Rechallenging subjects with occupational asthma due to toluene diisocyanate (TDI), after long-term removal from exposure. International Archives of Occupational and Environmental Health 80 (4), 298-305. DOI: 10.1007/s00420-006-0134-3 (last accessed 2016-09-20)

Plitnick L.M., Loveless S.E., Ladics G.S., Holsapple M.P., Smialowicz R.J., Woolhiser M.R., Anderson P.K., Smith C., and Selgrade M.J.K. (2005): Cytokine mRNA profiles for isocyanates with known and unknown potential to induce respiratory sensitization. Toxicology 207 (3), 487-499. DOI: 10.1016/j.tox.2004.11.001 (last accessed 2016-09-20)

Pollaris L., Devos F., de Vooght V., Seys S., Nemery B., Hoet P.M., and Vanoirbeek J.J. (2015): Toluene diisocyanate and methylene diphenyl diisocyanate: Asthmatic response and cross-reactivity in a mouse model. Archives of Toxicology, 1-9. DOI: 10.1007/s00204-015-1606-6 (last accessed 2016-09-20)

Porter C.V., Higgins R.L., and Scheel L.D. (1975): A retrospective study of clinical, physiologic and immunologic changes in workers exposed to toluene diisocyanate. American Industrial Hygiene Association Journal 36 (3), 159-168. DOI: 10.1080/0002889758507230 (last accessed 2016-09-20)

Pourabedian S., Barkhordari A., Habibi E., Rismanchiyan M., and Zare M. (2010): Effect of 1,6-hexamethylene diisocyanate exposure on peak flowmetry in automobile paint shop workers in Iran. Archives of Industrial Hygiene and Toxicology 61 (2), 183-189. DOI: 10.2478/10004-1254-61-2010-1994 (last accessed 2016-09-28)

Pronk A., Preller L., Doekes G., Wouters I.M., Rooijackers J., Lammers J.W., and Heederik D. (2009): Different respiratory phenotypes are associated with isocyanate exposure in spray painters. European Respiratory Journal 33 (3), 494-501. DOI: 10.1183/09031936.00091408 (last accessed 2016-09-20)

Pronk A., Preller L., Raulf-Heimsoth M., Jonkers I.C., Lammers J.W., Wouters I.M., Doekes G., Wisnewski A.V., and Heederik D. (2007): Respiratory symptoms, sensitization, and exposure response relationships in spray painters exposed to isocyanates. American Journal of Respiratory and Critical Care Medicine 176 (11), 1090-1097. DOI: 10.1164/rccm.200702-215OC (last accessed 2016-09-20)

Rattray N.J., Botham P.A., Hext P.M., Woodcock D.R., Fielding I., Dearman R.J., and Kimber I. (1994): Induction of respiratory hypersensitivity to diphenylmethane-4,4'-diisocyanate (MDI) in guinea pigs. Influence of route of exposure. Toxicology 88, 1-3. DOI: 10.1016/0300-483X(94)90108-2 (last accessed 2016-09-20)

Reh C.M. and Lushniak B.D. (1984): Health Hazard Evaluation Report No. HETA-87-350-2084. Trailmobile, Inc., Charleston, Illinois. HETA 87-350-2084. Hazard Evaluations and Technical Assistance Branch. National Institute for Occupational Safety and Health (NIOSH), Cincinnati, Ohio, USA

Reuzel P.G., Arts J.H., Lomax L.G., Kuijpers M.H., Kuper C.F., Gembardt C., Feron V.J., and Loser E. (1994a): Chronic inhalation toxicity and carcinogenicity study of respirable polymeric methylene diphenyl diisocyanate (polymeric MDI) aerosol in rats. Fundamental and Applied Toxicology 22 (2), 195-210. DOI: 10.1006/faat.1994.1024 (last accessed 2016-09-20)

Reuzel P.G.J., Kuper C.F., Feron V.J., Appelman L.M., and Loser E. (1994b): Acute, subacute, and subchronic inhalation toxicity studies of respirable polymeric methylene dipheyl diisocyanate (polymeric MDI) aerosol in rats. Fundamental and Applied Toxicology 22 (2), 186-194. DOI: 10.1006/faat.1994.1023 (last accessed 2016-09-20)

Safepharm (1992): XDI: 1,3-Benzenebis(methyl isocyanate): Magnusson & Kligman maximisation study in the guinea pig. Project no. 458/13, date: 1992-09-28. Safepharm Laboratories Ltd. Mitsui Toatsu Chemicals Inc., unpublished

Safepharm (1998a): TODI: Magnusson & Kligman maximisation study in the guinea pig. Report no.: 235/224, date: 1998-02-26. Safepharm Laboratories Ltd. Nippon Soda Co. Ltd., unpublished

Safepharm (1998b): XDI (MR-6A): Magnusson & Kligman maximisation study in the guinea pig. Project No. 458/195, date: 1998-04-28. Safepharm Laboratories Ltd. Mitsui Toatsu Chemicals Inc., unpublished

Sales J.H. and Kennedy K.S. (1990): Epiglottic dysfunction after isocyanate inhalation exposure. Archives of Otolaryngology - Head and Neck Surgery 116 (6), 725-727. DOI: 10.1001/archotol.1990.01870060083016 (last accessed 2016-09-28)

Sangha G.K. and Alarie Y. (1979): Sensory irritation by toluene diisocyanate in single and repeated exposures. Toxicology and Applied Pharmacology 50, 533-547. DOI: 10.1016/0041-008X(79)90408-3 (last accessed 2016-09-20)

Sangha G.K., Matijak M., and Alarie Y. (1981): Comparison of some mono- and diisocyanates as sensory irritants. Toxicology and Applied Pharmacology 57 (2), 241-246. DOI: 10.1016/0041-008X(81)90285-4 (last accessed 2016-09-20)

Satoh T., Kramarik J.A., Tollerud D.J., and Karol M.H. (1995): A murine model for assessing the respiratory hypersensitivity potential of chemical allergens. Toxicology Letters 78 (1), 57-66. DOI: https://doi.org/10.1016/0378-4274(94)03234-X

Scheerens H., Buckley T.L., Muis T., van Loveren H., and Nijkamp F.P. (1996): The involvement of sensory neuropeptides in toluene diisocyanate-induced tracheal hyperreactivity in the mouse airways. British Journal of Pharmacology 119 (8), 1665-1671. DOI: 10.1111/j.1476-5381.1996.tb16087.x (last accessed 2016-09-20)

Scheerens H., Buckley T.L., Muis T.L., Garssen J., Dormans J., Nijkamp F.P., and van Loveren H. (1999): Long-term topical exposure to toluene diisocyanate in mice leads to antibody production and in vivo airway hyperresponsiveness three hours after intranasal challenge. American Journal of Respiratory and Critical Care Medicine 159 (4), 1074-1080. DOI: 10.1164/ajrccm.159.4.9701012 (last accessed 2016-09-20)

Schwarze J., Cieslewicz G., Hamelmann E., Joetham A., Shultz L.D., Lamers M.C., and Gelfand E.W. (1999): IL-5 and Eosinophils Are Essential for the Development of Airway Hyperresponsiveness Following Acute Respiratory Syncytial Virus Infection. The Journal of Immunology 162 (5), 2997-3004. http://www.jimmunol.org/content/jimmunol/162/5/2997.full.pdf

Selgrade M.K., Boykin E.H., Haykal-Coates N., Woolhiser M.R., Wiescinski C., Andrews D.L., Farraj A.K., Doerfler D.L., and Gavett S.H. (2006): Inconsistencies between cytokine profiles, antibody responses, and respiratory hyperresponsiveness following dermal exposure to isocyanates. Toxicological Sciences 94 (1), 108-117. DOI: 10.1093/toxsci/kfl094 (last accessed 2016-09-20)

Song J., Zhao H., Dong H., Zhang D., Zou M., Tang H., Liu L., Liang Z., Lv Y., Zou F., and Cai S. (2013): Mechanism of E-cadherin redistribution in bronchial airway epithelial cells in a TDI-induced asthma model. Toxicology Letters 220 (1), 8-14. DOI: 10.1016/j.toxlet.2013.03.033 (last accessed 2016-09-20)

Stadler J. and Karol M.H. (1984): Experimental delayed hypersensitivity following inhalation of dicyclohexylmethane-4,4′-diisocyanate: A concentration-response relationship. Toxicology and Applied Pharmacology 74 (2), 244-249. DOI: 10.1016/0041-008X(84)90149-2 (last accessed 2016-09-20)

Stadler J.C. and Karol M.H. (1985): Use of dose-response data to compare the skin sensitizing abilities of dicyclohexylmethane-4,4′-diisocyanate and picryl chloride in two animal species. Toxicology and Applied Pharmacology 78 (3), 445-450. DOI: 10.1016/0041-008X(85)90251-0 (last accessed 2016-09-20)

Stern M.L., Brown T.A., Brown R.D., and Munson A.E. (1989): Contact hypersensitivity response to isophorone diisocyanate in mice. Drug and Chemical Toxicology 12 (3-4), 287-296. DOI: 10.3109/01480548908999159 (last accessed 2016-09-20)

Stingeni L., Bellini V., and Lisi P. (2008): Occupational airborne contact urticaria and asthma: simultaneous immediate and delayed allergy to diphenylmethane-4,4'-diisocyanate. Contact Dermatitis 58 (2), 112-113. DOI: 10.1111/j.1600-0536.2007.01180.x (last accessed 2016-09-20)

Sun L.Z., Elsayed S., Bronstad A.M., van Do T., Irgens A., Aardal N.P., and Aasen T.B. (2007): Airway inflammation and bronchial remodelling in toluene diisocyanate-exposed BALB/c mouse model. Scandinavian Journal of Immunology 65 (2), 118-125. DOI: 10.1111/j.1365-3083.2006.01882.x (last accessed 2016-09-20)

Svensson-Elfsmark L., Koch B.L., Gustafsson A., and Bucht A. (2009): Rats repeatedly exposed to toluene diisocyanate exhibit immune reactivity against methyl isocyanate-protein conjugates. International Archives of Allergy and Immunology 150 (3), 229-236. DOI: 10.1159/000222675 (last accessed 2016-09-20)

Swensson A., Holmquist C.E., and Lundgren K.D. (1955): Injury to the respiratory tract by isocyanates used in making lacquers. British Journal of Industrial Medicine 12 (1), 50-53. DOI: 10.1136/oem.12.1.50 (last accessed 2016-09-20)

Swierczynska-Machura D., Nowakowska-Swirta E., Walusiak-Skorupa J., Piasecka-Zelga J., Swiercz R., Nocun M., and Palczynski C. (2014): Effect of inhaled toluene diisocyanate on local immune response based on murine model for occupational asthma. Journal of Immunotoxicology 11 (2), 166-171. DOI: 10.3109/1547691x.2013.818745 (last accessed 2016-09-20)

Swierczynska-Machura D., Walusiak-Skorupa J., Nowakowska-Swirta E., Piasecka-Zelga J., Swiercz R., and Palczynski C. (2012): Immunological determinants in a murine model of toluene diisocyanate-induced asthma. International Journal of Occupational and Environmental Health 25 (4), 492-498. DOI: 10.2478/s13382-012-0063-1 (last accessed 2016-09-20)

Tanaka K., Kawai M., and Maekawa N. (1983): Experimental model of asthma by toluene diisocyanate (TDI). Bulletin of the Chest Disease Research Institute of the Kyoto University 16, 1-9. http://hdl.handle.net/2433/52154 (last accessed 2016-09-20)

Tanaka K.I. (1980): Contact sensitivity in mice induced by tolylene diisocyanate (TDI). Journal of Dermatology 7 (4), 277-280. DOI: 10.1111/j.1346-8138.1980.tb01969.x (last accessed 2016-09-20)

Tanaka K.I., Takeoka A., Nishimura F., and Hanada S. (1987): Contact sensitivity induced in mice by methylene bisphenyl diisocyanate. Contact Dermatitis 17 (4), 199-204. DOI: 10.1111/j.1600-0536.1987.tb02713.x (last accessed 2016-09-20)

Tarkowski M., Vanoirbeek J.A., Vanhooren H.M., de Vooght V., Mercier C.M., Ceuppens J., Nemery B., and Hoet P.H. (2007): Immunological determinants of ventilatory changes induced in mice by dermal sensitization and respiratory challenge with toluene diisocyanate. American Journal of Physiology. Lung Cellular and Molecular Physiology 292 (1), L207-214. DOI: 10.1152/ajplung.00157.2005 (last accessed 2016-09-20)

Tarlo S.M., Liss G.M., Dias C., and Banks D.E. (1997): Assessment of the relationship between isocyanate exposure levels and occupational asthma. American Journal of Industrial Medicine 32 (5), 517-521. DOI: 10.1002/(SICI)1097-0274(199711)32:5<517::AID-AJIM12>3.0.CO;2-5 (last accessed 2016-09-19)

Thorne P.S., Hillebrand J.A., Lewis G.R., and Karol M.H. (1987): Contact sensitivity by diisocyanates: Potencies and cross-reactivities. Toxicology and Applied Pharmacology 87 (1), 155-165. DOI: 10.1016/0041-008X(87)90093-7 (last accessed 2016-09-20)

Tominaga M., Kohno S., Tanaka K.I., and Ohata K. (1985): Studies on toluene diisocyanate (TDI)-induced delayed type hypersensitivity. Japanese Journal of Pharmacology 39 (2), 163-171. DOI: 10.1254/jjp.39.163 (last accessed 2016-09-20)

Tornling G., Alexandersson R., Hedenstierna G., and Plato N. (1990): Decreased lung function and exposure to diisocyanates (HDI and HDI-BT) in car repair painters: Observations on re-examination 6 years after initial study. American Journal of Industrial Medicine 17 (3), 299-310. DOI: 10.1002/ajim.4700170302 (last accessed 2016-09-28)

Tyl R.W., Neeper-Bradley T.L., Fisher L.C., Dodd D.E., Pritts I.M., Losco P.E., Lyon J.P., and Landry T.D. (1999): Two-generation reproductive toxicity study of inhaled toluene diisocyanate vapor in CD rats. Toxicological Sciences 52 (2), 258-268. DOI: 10.1093/toxsci/52.2.258 (last accessed 2016-09-28)

Ulvestad B., Melbostad E., and Fuglerud P. (1999): Asthma in tunnel workers exposed to synthetic resins. Scandinavian Journal Of Work Environment & Health 25 (4), 335-341. DOI: 10.5271/sjweh.443 (last accessed 2016-09-21)

Union Carbide (1988): Developmental toxicity study of inhaled toluene diisocyanate vapor in CD (Sprague-Dawley) rats. III report 10548, III project NA-AB-50, date: 1988-12-06. Union Carbide Chemicals and Plastics Company, Bushy Run Research Center. International Isocyanate Institute, unpublished

Union Carbide (1989): Two-generation reproduction study of inhaled toluene diisocyanate in CD (Sprague-Dawley) rats. III report 10608, III project NA-AB-50, date: 1989-03-17. Union Carbide Chemicals and Plastics Company, Bushy Run Research Center. International Isocyanate Institute, unpublished

Union Carbide (1990): CT-329-87 fourteen-week vapor inhalation study with rats and mice Report no. 51-579, company study no. 87-57-80009, date: 1990-01-18. Union Carbide Chemicals and Plastics Company, Bushy Run Research Center. Cytec Industries, unpublished

Union Carbide (1992): Evaluation of sensory irritation potential and assessment of the respiratory response during exposure to meta-tetramethylxylene diisocyanate (CT-368-88) vapor. Report no. 52-506, date: 1992-05-22. Union Carbide Chemicals and Plastics Company, Bushy Run Research Center. Cytec Industries, unpublished

University of Louisville (1987): Preparation and immunologic evaluation of allergenic hapten-protein conjugates, date: 1983-11-23. American Cyanamide Co., unpublished

Valks R., Conde-Salazar L., and Barrantes O.L. (2003): Occupational allergic contact urticaria and asthma from diphenylmethane-4,4'-diisocyanate. Contact Dermatitis 49 (3), 166-167. DOI: 10.1111/j.0105-1873.2003.0185j.x (last accessed 2016-09-21)

van Och F.M.M., Slob W., de Jong W.H., Vandebriel R.J., and van Loveren H. (2000): A quantitative method for assessing the sensitizing potency of low molecular weight chemicals using a local lymph node assay: Employment of a regression method that includes determination of the uncertainty margins. Toxicology 146 (1), 49-59. DOI: 10.1016/S0300-483X(00)00165-7 (last accessed 2016-09-21)

Vandebriel R.J., de Jong W.H., Spiekstra S.W., van Dijk M., Fluitman A., Garssen J., and van Loveren H. (2000): Assessment of preferential T-helper 1 or T-helper 2 induction by low molecular weight compounds using the local lymph node assay in conjunction with RT-PCR and ELISA for interferon-gamma and interleukin-4. Toxicology and Applied Pharmacology 162 (2), 77-85. DOI: 10.1006/taap.1999.8841 (last accessed 2016-09-21)

Vandenplas O., Cartier A., Lesage J., Cloutier Y., Perreault G., Grammer L.C., Shaughnessy M.A., and Malo J.-L. (1993a): Prepolymers of hexamethylene diisocyanate as a cause of occupational asthma. Journal of Allergy and Clinical Immunology 91 (4), 850-861. DOI: 10.1016/0091-6749(93)90342-D (last accessed 2016-09-28)

Vandenplas O., Cartier A., Lesage J., Perrault G., Grammer L.C., and Malo J.L. (1992a): Occupational asthma caused by a prepolymer but not the monomer of toluene diisocyanate (TDI). Journal of Allergy and Clinical Immunology 89 (6), 1183-1188. DOI: 10.1016/0091-6749(92)90303-J (last accessed 2016-09-28)

Vandenplas O., Malo J.L., Cartier A., Perreault G., and Cloutier Y. (1992b): Closed-circuit methodology for inhalation challenge tests with isocyanates. American Review of Respiratory Disease 145 (3), 582-587. DOI: 10.1164/ajrccm/145.3.582 (last accessed 2016-09-21)

Vandenplas O., Malo J.L., Dugas M., Cartier A., Desjardins A., L,vesque J., Shaughnessy M.A., and Grammer L.C. (1993b): Hypersensitivity pneumonitis-like reaction among workers exposed to diphenylmethane diisocyanate (MDI). American Review of Respiratory Disease 147 (2), 338-346. DOI: 10.1164/ajrccm/147.2.338 (last accessed 2016-09-28)

Vanoirbeek J.A.J., de Vooght V., Synhaeve N., Nemery B., and Hoet P.H.M. (2009): Is toluene diamine a sensitizer and is there cross-reactivity between toluene diamine and toluene diisocyanate? Toxicological Sciences 109 (2), 256-264. DOI: 10.1093/toxsci/kfp065 (last accessed 2016-09-21)

Vanoirbeek J.A.J., Tarkowski M., Ceuppens J.L., Verbeken E.K., Nemery B., and Hoet P.H.M. (2004): Respiratory response to toluene diisocyanate depends on prior frequency and concentration of dermal sensitization in mice. Toxicological Sciences 80 (2), 310-321. DOI: 10.1093/toxsci/kfh155 (last accessed 2016-09-21)

Venables K.M., Dally M.B., Burge P.S., Pickering C.A., and Newman Taylor A.J. (1985): Occupational asthma in a steel coating plant. British Journal of Industrial Medicine 42 (8), 517-524. DOI: 10.1136/oem.42.8.517 (last accessed 2016-09-28)

Wang J.D., Huang P.H., Lin J.M., Su S.Y., and Wu M.C. (1988): Occupational asthma due to toluene diisocyanate among velcro-like tape manufacturers. American Journal of Industrial Medicine 14 (1), 73-78. DOI: 10.1002/ajim.4700140109 (last accessed 2016-09-29)

Wegman D.H., Musk A.W., Main D.M., and Pagnotto L.D. (1982): Accelerated loss of FEV-1 in polyurethane production workers: A four-year prospective study. American Journal of Industrial Medicine 3 (2), 209-215. DOI: 10.1002/ajim.4700030212 (last accessed 2016-09-21)

Wegman D.H., Pagnotto L.D., Fine L.J., and Peters J.M. (1974): A dose response relationship in TDI workers. Journal of Occupational Medicine 16 (4), 258-260. http://journals.lww.com/joem/Abstract/1974/04000/A_Dose_Response_Relationship_in_TDI_Workers_.6.aspx (last accessed 2016-09-21)

Wegman D.H., Peters J.M., Pagnotto L., and Fine L.J. (1977): Chronic pulmonary function loss from exposure to toluene diisocyanate. British Journal of Industrial Medicine 34 (3), 196-200. DOI: 10.1136/oem.34.3.196 (last accessed 2016-09-21)

Weill H., Butcher B., Dharmarajan V., Glindmeyer H.W., Jones R.N., Carr J., O'Neill C., and Salvaggio J. (1981): Respiratory and immunologic evaluation of isocyanate exposure in a new manufacturing plant.

NIOSH Publication No. 81-125. National Institute for Occupational Safety and Health (NIOSH). U.S. Government Printing Office

Weyel D.A., Rodney B.S., and Alarie Y. (1982): Sensory irritation, pulmonary irritation, and acute lethality of a polymeric isocyanate and sensory irritation of 2,6-toluene diisocyanate. Toxicology and Applied Pharmacology 64 (3), 423-430. DOI: 10.1016/0041-008X(82)90238-1 (last accessed 2016-09-21)

Weyel D.A. and Schaffer R.B. (1985): Pulmonary and sensory irritation of diphenylmethane-4,4′- and dicyclohexylmethane-4,4′-diisocyanate. Toxicology and Applied Pharmacology 77 (3), 427-433. DOI: 10.1016/0041-008X(85)90182-6 (last accessed 2016-09-21)

Williamson K.S. (1964): Studies of diisocyanate workers (1). Occupational Medicine 14 (1), 81-88. DOI: 10.1093/occmed/14.1.81 (last accessed 2016-09-21)

Williamson K.S. (1965): Studies of diisocyanate workers. (2). Occupational Medicine 15 (1), 29-35. DOI: 10.1093/occmed/15.1.29 (last accessed 2016-09-28)

Wisnewski A.V., Liu J., and Colangelo C.M. (2015): Glutathione reaction products with a chemical allergen, methylene-diphenyl diisocyanate, stimulate alternative macrophage activation and eosinophilic airway inflammation. Chemical Research in Toxicology 28 (4), 729-737. DOI: 10.1021/tx5005002 (last accessed 2016-09-21)

Woodbury J.W. (1956): Asthmatic syndrome following exposure to toluene diisocyanate. Industrial Medicine & Surgery 25, 540-543. https://www.ncbi.nlm.nih.gov/pubmed/13366472 (last accessed 2016-10-07)

Woolhiser M., Stephenson A., and Boverhof D. (2013): Sensitization potential of low-monomer diisocyanate prepolymers. http://msdssearch.dow.com/PublishedLiteratureDOWCOM/dh_0869/0901b803808699bf.pdf (last accessed 2016-09-21)

Woolhiser M.R., Hayes B.B., and Meade B.J. (1998): A combined murine local lymph node and irritancy assay to predict sensitization and irritancy potential of chemicals. Toxicology Methods 8 (4), 245-256. DOI: 10.1080/105172398242835 (last accessed 2016-09-21)

Yamada K., Amitani R., Niimi A., and Kuze F. (1995): Interstitial pneumonitis-like lesions in guinea-pigs following repeated exposure to toluene diisocyanate. European Respiratory Journal 8 (8), 1300-1306. http://erj.ersjournals.com/content/8/8/1300.long (last accessed 2016-09-21)

Zeiss C.R., Kanellakes T.M., Bellone J.D., Levitz D., Pruzansky J.J., and Patterson R. (1980): Immunoglobulin E-mediated asthma and hypersensitivity pneumonitis with precipitating anti-hapten antibodies due to diphenylmethane diisocyanate (MDI) exposure. Journal of Allergy and Clinical Immunology 65 (5), 347-352. DOI: 10.1016/0091-6749(80)90211-0 (last accessed 2016-09-21)

Zheng K.C., Ariizumi M., Todoriki H., and Shinjo M. (1998): Cytokine production by splenocytes and thymocytes in mice after intranasal exposure to toluene diisocyanate. Journal of Occupational Health 40 (4), 279-284. http://joh.sanei.or.jp/pdf/E40/E40_4_05.pdf (last accessed 2016-09-21)

Zheng K.C., Nong D.X., Morioka T., Todoriki H., and Ariizumi M. (2001): Elevated interleukin-4 and interleukin-6 in rats sensitized with toluene diisocyanate. Industrial Health 39 (4), 334-339. DOI: 10.2486/indhealth.39.334 (last accessed 2016-09-21)

Zissu D., Binet S., and Limasset J.C. (1998): Cutaneous sensitization to some polyisocyanate prepolymers in guinea pigs. Contact Dermatitis 39 (5), 248-251. DOI: 10.1111/j.1600-0536.1998.tb05918.x (last accessed 2016-09-21)