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Toluene diisocyanates

MAK Value Documentation, supplement – Translation of the German version from 2021

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Abstract

The German Commission for the Investigation of Health Hazards of Chemical Compounds in the Work Area has re-evaluated toluene diisocyanates (2,4-toluene diisocyanate [584-84-9], 2,6-toluene diisocyanate [91-08-7] and the isomer mixture [26471-62-5]) to derive a maximum concentration at the workplace (MAK value) and to review its carcinogenicity classification. The critical effects are sensory irritation and sensitization in humans and animals. A concentration that protects from sensitization cannot be derived. Neither irritation nor loss of lung function in humans was described at a concentration of 1 µl/m^3. In some studies, however, lung function decrements were observed at a mean concentration of 1 µl/m^3; these were most likely caused by peak concentrations of 20 µl/m^3 or above. A LOAEC of 50 µl/m^3 was derived for chronic and necrotic rhinitis from a two-year inhalation study in rats and mice. A benchmark dose lower confidence limit of 10 µl/m^3 was calculated from the rat data for a 5% increase (BMDL05) in chronic rhinitis. A BMDL for mice could not be calculated. Based on the BMDL05, a MAK value of 1 µl/m^3 has been set taking into account the extrapolation to humans (1:3), the higher sensitivity of mice, the experience at the workplace and the "Preferred Value Approach". Toluene diisocyanates are not genotoxic after inhalation and genotoxic doses such as after oral exposure are not achieved due to the irritant effect. Toluene diisocyanates were not carcinogenic in an inhalation study in rats and mice and it was estimated that the carcinogenic toluenediamine is formed only in very low amounts from toluene diisocyanates during exposure at the level of the MAK value of 1 µl/m^3. As the critical effect is sensory irritation, Peak Limitation Category I has been assigned. Toluene diisocyanates are potent sensitizers; therefore, in analogy to other diisocyanates, an excursion factor of 1 is set. Irritation of the respiratory tract at a mean concentration of 5 µl/m^3 is caused by exposure peaks; therefore, a momentary value of 5 µl/m^3 is derived to avoid very high short-term concentrations. Toluene diisocyanates are assigned to Pregnancy Risk Group C because the margin between the NOAEC for developmental toxicity in rats and the MAK value is sufficient and no teratogenicity was observed. Due to the skin and respiratory sensitizing effects, the designations with "Sa" and "Sh" are confirmed. The systemic availability of the substance or its active metabolite toluenediamine after dermal exposure seems to be considerably reduced by the high reactivity of toluene diisocyanates and their affinity with structural components of the skin. There is no indication of systemic effects after

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dermal exposure. Therefore, toluene diisocyanates are not expected to be taken up via the skin in toxicologically relevant amounts.

MAK value (2020)	**0.001 ml/m³ (ppm) ≙ 0.007 mg/m³**
Peak limitation (2020)	**Category I, excursion factor 1**
Momentary value (2020)	**0.005 ml/m³ (ppm) ≙ 0.035 mg/m³**
Absorption through the skin	–
Sensitization (2014)	**Sah**
Carcinogenicity	–
Prenatal toxicity (2020)	**Pregnancy Risk Group C**
Germ cell mutagenicity	–
BAT value (2020)	**5 µg Σ 2,4-toluenediamine and 2,6-toluenediamine (after hydrolysis)/g creatinine**
CAS number	2,4-toluene diisocyanate [584-84-9]
	2,6-toluene diisocyanate [91-08-7]
	isomer mixture [26471-62-5]
Vapour pressure	2,4-toluene diisocyanate: 0.011 hPa at 20 °C (NCBI 2020 a)
	2,6-toluene diisocyanate: 0.028 hPa at 25 °C (NCBI 2020 b)
	isomer mixture: no data
1 ml/m³ (ppm) ≙ 7.24 mg/m³	**1 mg/m³ ≙ 0.138 ml/m³ (ppm)**

Note: The substance can occur as vapour and aerosol.

In 2000, the 2,4-isomer and 2,6-isomer of toluene diisocyanate (TDI) and mixtures of both isomers were classified in Carcinogen Category 3 A (Greim 1999, available in German only; Greim 2003). Furthermore, documentation and a supplement were published for the sensitizing effects of the substances (Hartwig 2013; 2015, available in German only). As new data have become available, this supplement re-evaluates the classification and reviews whether a MAK value can be derived.

The isomer mixture is generally composed of 2,4-TDI and 2,6-TDI in a ratio of either 80% : 20% or 65% : 35%. For each study described below, the ratio of 2,4-TDI to 2,6-TDI is given.

Formation of 2,4-toluenediamine (TDA) and 2,6-TDA from 2,4-TDI and 2,6-TDI in the air

In a study that investigated the formation of TDA from 2,4-TDI and 2,6-TDI in the air, at TDI concentrations of 0.36 mg/m³ to 4.3 mg/m³ (temperature: 27 °C; humidity: 7%–70%) 2,4-TDA or 2,6-TDA was not observed (Holdren et al. 1984). The 2,4-TDA and 2,6-TDA concentrations in generated 2,4-TDI and 2,6-TDI atmospheres were also determined. 2,4-TDA or 2,6-TDA was not detected at 2,4-TDI and 2,6-TDI concentrations of 20 to 50 µg/m³. The limit of detection was 0.2 to 0.5 µg/m³ (Brorson et al. 1989). Likewise, in a volunteer study, no 2,4-TDA or 2,6-TDA (limit of detection: 0.5 µg/m³) was found in the air during exposure to 40 µg/m³ of 2,4-TDI and 2,6-TDI (Skarping et al. 1991).

A saturated vapour atmosphere at room temperature contains about 20 ml/m³ of 2,4-TDI and 2,6-TDI.

Methods of analysis

A number of different methods are available for the determination of isocyanates. The analysis by paper tape monitor is based on colour changes occurring on a filter material impregnated with special substances; the air from the exposure chamber is passed through this filter at a constant flow rate. The intensity of the colour change is proportional to the concentration of isocyanate groups in the air (NCO sum parameter). This method is not suitable for the determination of aerosols. Until about 2014, the method was used in many workplace studies. A study investigated the reliability of 2 different paper tape monitors that were in use until 2014 by applying a validated discontinuous procedure (IFA method 7670) with the isocyanates hexamethylene diisocyanate (HDI) and diphenylmethane-4,4′-diisocyanate (MDI). After comparing the results determined by the 2 paper tape methods with the levels detected by the IFA method, it was found that the concentrations were too low (Monsé et al. 2015). At a concentration of about 5 µl/m^3, the deviation is negligible; however, at levels > 5 µl/m^3, the actual concentrations are underestimated.

Therefore, the concentrations determined in the air in the studies of Clark et al. (1998, 2003) were probably too low. As the deviations up to a concentration of 5 µl/m^3 can be regarded as negligible and all concentrations that were found to be above 5 µl/m^3 were in fact probably higher, the derived MAK value offers the workers protection irrespective of the method of analysis used, and a conversion factor is not required.

1 Toxic Effects and Mode of Action

2,4-TDI, 2,6-TDI and mixtures of the 2 isomers cause irritation and sensitization of the skin and respiratory tract in humans and animals; from the standpoint of occupational medicine, the sensitizing effects on the airways are the most critical effects. However, high or prolonged dermal exposure may also induce or contribute to respiratory tract sensitization. In addition, 2,4-TDI, 2,6-TDI and their mixtures may cause non-specific bronchial hyperreactivity and, in rare cases, allergic alveolitis (Greim 1999; Hartwig 2015). A LOAEC (lowest observed adverse effect concentration) of 50 µl/m^3 for chronic and necrotizing rhinitis was derived from 2-year inhalation studies in rats and mice.

The available in vivo studies of genotoxicity did not demonstrate clastogenic effects in the lungs or bone marrow, and DNA adducts or unscheduled DNA repair synthesis (UDS) were not induced in the liver of rats. Studies that investigated mutagenicity in mammals are not available. A re-evaluation of the carcinogenicity induced by inhalation exposure of rats and mice did not yield evidence that the test substance led to an increase in the tumour incidence. Oral exposure caused fibromas, fibrosarcomas, pancreatic tumours and neoplastic nodules in the liver in rats and haemangiosarcomas and liver tumours in mice. Thus, the tumour spectrum is similar to that observed after exposure to 2,4-TDA.

In a prenatal developmental toxicity study in Sprague Dawley rats exposed by inhalation from days 6 to 15 of gestation, the incidence of incomplete ossification of the 5th cervical vertebra was increased at a TDI concentration of 500 µl/m^3 with concurrent maternal toxicity. In a 2-generation study in Sprague Dawley rats with inhalation exposure, perinatal toxicity was not observed up to the highest TDI concentration tested of 300 µl/m^3.

2 Mechanism of Action

The effects of TDI are determined by the reactivity of the isocyanate groups. The reactivity is responsible for both local irritation and the property of TDI to form haptens leading to secondary immunological reactions. Accordingly, TDI has the potential to cause irritation of the skin and eyes and sensitization of the skin and airways (asthmagenic potential). However, the conjugates responsible for the allergic reactions are still largely unknown (see also Greim 1999; Hartwig 2013, 2015).

2.1 Sensitizing effects on the airways

Bronchial asthma is a relatively common syndrome ("isocyanate asthma") induced by diisocyanates such as the 2,4-TDI/2,6-TDI mixture. Pathogenetically, both immunological and non-immunological mechanisms may be involved; the immunological mechanisms are mediated by IgE only in some cases. The evidence of specific IgE antibodies against TDI conjugates does not correlate well with the clinical symptoms. Isocyanate asthma is generally accompanied by non-specific bronchial hypersensitivity (see Greim 1999); this hypersensitivity may vary markedly in its severity, both interindividually and intraindividually, and may occur even before asthma symptoms are manifest. Accordingly, the incidences of non-specific airway reactivity were increased in workers exposed to TDI. The induction of isocyanate sensitization depends on the dose and the individual. The role of peak exposures is not yet fully understood. Several animal models have shown that sensitization of the airways is induced by skin contact; therefore, this is being discussed as a mechanism. In general, the total dose, rather than the concentration or duration alone, is decisive for the induction of an asthmatic reaction (Vandenplas et al. 1999; see Greim 1999).

$CD4^+$ and $CD8^+$ T-lymphocytes were detected in the bronchoalveolar lavage fluid (BALF), sputum or biopsy material of persons with asthma induced by diisocyanates. The levels of cytokines, such as TNF-α, interferon-γ and the interleukins IL-1β and IL-8 (IL), indicating a Th1-mediated reaction, and IL-4, IL-5, IL-6 or IL-15, indicating a Th2-mediated reaction, were increased (Maestrelli et al. 1994, 1997; Piirilä et al. 2008). Other studies found increased sputum levels of neutrophils or eosinophils (Lemière et al. 2002; Saetta et al. 1992), myeloperoxidase (Park et al. 1999) and metalloproteinase-9 (MMP-9; gelatinase B) (Park et al. 2003). However, also reduced levels of MMP-9 in the BALF of patients with diisocyanate-induced asthma were reported (Piirilä et al. 2010). Increased levels of neutrophils, of various interleukins and of changed matrix proteins in the BALF or sputum suggest inflammatory processes in the lungs.

Leukotriene B4 was detected in persons with a delayed reaction to a 2,4-TDI/2,6-TDI mixture in the bronchial provocation test. Leukotriene B4 is an inflammatory leukotriene that forms from neutrophilic granulocytes and mononuclear phagocytes (Zocca et al. 1990). In addition, increases in serum periostin levels that correlated with the extent of non-specific bronchial hyperreactivity (Lee et al. 2018) and increases in serum folliculin (Pham et al. 2017) were reported. Increased transferrin and reduced haem oxygenase-1/ferritin levels were found in the BALF and serum of persons with TDI asthma. TDI suppresses the translocation of Nrf2 and its binding to the ARE region of the haem oxygenase 1-promoter by inhibiting the phosphorylation of mitogen-activated protein kinases (MAPK) (Kim et al. 2010).

A number of studies in mice demonstrated that IL-4, several other interleukins and interferon-γ were secreted (for example Fukuyama et al. 2008; Johnson et al. 2007; Matheson et al. 2005 b; Tarkowski et al. 2007, 2008; Vandebriel et al. 2000; Vanoirbeek et al. 2008), whereas other studies reported that only or mainly Th2 cytokines were expressed (Plitnick et al. 2005; Selgrade et al. 2006).

A study in mice with dermal induction and nasal provocation found that the allergic reaction was mediated by the B lymphocytes. However, IgE and T lymphocytes or the cytokines that they secreted were not involved (De Vooght et al. 2013; Haenen et al. 2015).

In CD4 knockout mice that were first sensitized to the 2,4-TDI/2,6-TDI mixture and then challenged with the 2,4-TDI/2,6-TDI mixture, a decrease in hyperreactivity was observed that was statistically significant compared with that induced in wild-type mice. The IL-4 and IL-5 cytokines, interferon-γ and TNF-α were not increased in the CD4 knockout mice (Matheson et al. 2005 a).

These findings suggest a complex mechanism of action for the development of occupational asthma induced by TDI; it differs from the general mechanisms leading to the sensitizing effects on the airways.

Formation of (protein) adducts and antigenic structures

Inhaled diisocyanates are deposited in the airways and react with nucleophiles there. Quantitatively, glutathione is the main nucleophile in the lungs (Cantin et al. 1987; Rahman et al. 1999). The products of these reactions, diisocyanate mono-glutathione or bis-glutathione conjugates, are absorbed and may form albumin and haemoglobin adducts in the blood. This modification of proteins is induced by the transcarbamoylation of the thiocarbamates formed primarily with glutathione (Day et al. 1997; Lange et al. 1999 a; Wisnewski et al. 2011) rather than by the free amine, as was

previously assumed. Antigenic structures may form not only by the coupling of diisocyanates to soluble proteins such as albumin and haemoglobin (Mhike et al. 2016), but also by the reaction with proteins or cellular components of the lung fluid (see Greim 1999) or with structural proteins, such as tubulin (Lange et al. 1999 b) and keratin-18 (Ye et al. 2006). In vitro, TDI (no other details) stimulates the secretion of mucus by bronchial epithelial cells and rapidly decreases the intracellular glutathione level (Lantz et al. 2001).

In a human bronchial epithelial cell line incubated with HDI, a marked decrease in albumin binding and cytotoxicity was observed in vitro, the extent of which depended on the glutathione concentration that was added. Therefore, glutathione protects the cells from the binding of proteins with HDI and HDI-induced cell death, while glutathione depletion or the addition of oxidized glutathione led to impaired function and increased cytotoxicity (Wisnewski et al. 2005).

2.2 Sensory irritation

To some extent, the irritation induced by 2,4-TDI and 2,6-TDI is due to the activation of sensory nerve fibres that innervate the upper airways. The non-selective cation channel TRPA1 (transient receptor potential ankyrin 1, subfamily A) is decisive here. In CHO (a cell line derived from Chinese hamster ovary) and HEK293 (human embryonic kidney) cells in vitro, 2,4-TDI activated the TRPA1 receptor, but not the TRPV1 receptor (transient receptor potential cation channel, subfamily V, member 1; vanilloid receptor 1 (VR1); capsaicin receptor) (Devos et al. 2016; Taylor-Clark et al. 2009). In vivo studies confirmed these selective effects with TRPA1 knockout mice (Taylor-Clark et al. 2009). In these animals, the breathing frequency was not reduced after acute inhalation of a 1% 2,4-TDI aerosol. In the wild-type mice, the duration of braking (TB) was extended, which is typical of sensory irritation (Alarie 1998); this leads to the characteristic decrease in breathing frequency (Taylor-Clark et al. 2009). Using C57Bl/6 mice (wild-type) and TRPA1 and TRPV1 knockout mice that had been sensitized to 2,4-TDI, Devos et al. (2016) showed that there is a relationship between the cation channels TRPA1 and TRPV1 and the airway hyperreactivity induced by 2,4-TDI. After provocation with 2,4-TDI by inhalation in the methacholine test, the airway resistance (Raw) was increased only in the sensitized wild-type mice. Likewise, wild-type mice pre-treated with substance P or a TRPA1 blocker, did not develop airway hyperreactivity (Devos et al. 2016). Another study with mast cell-deficient mice showed that TDI-induced asthma may be due to mechanisms of interaction between the nervous system and immune system and that TRPA1 and TRPV1 channels and mast cells play a decisive role (Hox et al. 2013).

2.3 Carcinogenicity

Oral exposure induced fibromas, fibrosarcomas, pancreatic tumours and neoplastic nodules in the liver in rats and haemangiosarcomas and liver tumours in mice. These tumours are attributed to 2,4-TDA because 2,4-TDA makes up about 5% of the products formed after oral exposure to 2,4-TDI and the tumour spectrum is very similar to that after exposure to 2,4-TDA.

3 Toxicokinetics and Metabolism

3.1 Absorption, distribution, elimination

Absorption, distribution and elimination were described in detail in the 1999 supplement (Greim 1999) and data for the relationship between the external exposure and body burden were given in the addendum for the derivation of a BAT value (Leng et al. 2021, available in German only). Recent studies are described below.

A group of 18 patients with occupational asthma induced by diisocyanates was exposed to 2,4-TDI/2,6-TDI concentrations of 0.5 to 30 µl/m^3 for up to 120 minutes, and the 2,4-TDA/2,6-TDA concentrations were determined in the urine up to 24 hours after exposure. Depending on the level of inhalation exposure, the patients were divided into a group with low exposure of 496 µl/m^3 × minutes and a group with high exposure of 1596 µl/m^3 × minutes. The differences

between the 2,4-TDA/2,6-TDA concentrations determined in the urine of the 2 groups were only slight and not statistically significant. The urinary excretion half-life was 6 hours (Budnik et al. 2011).

The study of Timchalk et al. (1994) can be used to estimate the amount of 2,4-TDA that is formed after oral and inhalation exposure to 2,4-TDI. Male F344 rats were exposed head-only to a 2,4-(^{14}C)TDI concentration of 2 ml/m^3 for 4 hours. No free 2,4-TDA and only very small amounts of acetylated TDA were detected in the collected 12-hour urine (Table 1). Of the radioactive dose administered, 47% was recovered in the faeces and 15% was detected in the urine 48 hours after exposure. Acid-labile conjugates accounted for 90% of the quantified urinary metabolites. The half-life was reported to be 20 hours. After a 2,4-TDI dose of 60 mg/kg body weight was given by gavage, 81% of the radioactivity was detected in the faeces and 8% was found in the urine after 48 hours. Unlike after inhalation exposure, free 2,4-TDA was additionally detected in the urine of rats (Table 1). Acid-labile conjugates accounted for 65% of the quantified urinary metabolites. Other metabolites that were detected were monoacetylated TDA and diacetylated TDA. The half-life was 7.5 hours. In addition, the rats were given radioactively labelled 2,4-(^{14}C)TDA doses of 3 and 60 mg/kg body weight by gavage. The fraction of 2,4-TDA excreted with the urine was between 64% and 72% and the fraction excreted with the faeces was between 20% and 30%. The half-lives determined for 2,4-TDA were 8 hours (3 mg/kg body weight) and 4.6 hours (60 mg/kg body weight). The total distribution of the radioactivity was about the same after inhalation of 2 ml/m^3 for 4 hours (899 ± 35 µg eq TDI) as that after oral administration of a 2,4-TDA dose of 3 mg/kg body weight (729 µg eq TDA). About the same amount of free and acetylated TDA formed in total after oral administration of a 2,4-TDI dose of 60 mg/kg body weight and after oral administration of a 2,4-TDA dose of 3 mg/kg body weight (Table 1) (Timchalk et al. 1994).

Tab. 1 Formation of TDA after oral and inhalation exposure of male rats (Timchalk et al. 1994)

Exposure	µg TDA equivalents (radioactivity) in the urine		
	Free 2,4-TDA	Acetylated TDA	Sum of free and acetylated TDA
2 ml 2,4-TDI/m^3, 4 hours, inhalation	not detectable	0.26	0.26
60 mg 2,4-TDI/kg body weight, oral	2.08	13.29	15.37
60 mg 2,4-TDA/kg body weight, oral	183.48	454.89	638.37
3 mg 2,4-TDA/kg body weight, oral	3.93	16.22	20.15

After radioactively labelled 2,4-TDI (undiluted; dose: 11.6 mg/cm^2) was applied to the skin of 4 rats for 1 hour, 0.5% of the dose was found to have been absorbed (radioactivity in excreta, cage wash, plasma and carcass). A fraction of 0.03% of the dose was recovered in the plasma and urine of the animals. A flux of 58 µg/cm^2 and hour was calculated from the total amount absorbed. After the substance was washed off the skin, a residue of 7.5% of the applied dose remained around the application site. The authors carried out additional tests to analyse the recovery of dermally applied TDI from the skin surface after different exposure periods and found that the substance has a high reactivity and affinity for biological materials. In view of the low dermal absorption and high binding affinity, the authors concluded that the risk of systemic toxicity is generally low after dermal exposure (Hoffmann et al. 2010). After the exposure of a 2000 cm^2 surface area of skin (corresponding approximately to the area of both hands and forearms) to undiluted TDI for 1 hour, the total amount of TDI absorbed would be 116 mg based on the above-mentioned flux of 58 µg/cm^2 and hour.

A cotton patch (area: 15 cm^2) with 1.5 ml of a TDI solution (0.2%, 1% or 5% of a 2,4-TDI/2,6-TDI mixture (80% : 20%)) in olive oil was applied to the shaved dorsal skin of male SD rats for 5 hours (Yeh et al. 2008). This corresponds to 2.73, 13.65 and 68.25 mg TDI (sum of both isomers; density of olive oil used for calculation: 0.91 g/ml; applied amounts correspond to 15.68, 78.83 and 391.9 µmol (with a TDI molar mass of 174.16 g/mol)). Irritation of the skin was not observed. The amount of 2,4-TDA and 2,6-TDA excreted by the animals with the urine was monitored for 6 days from the beginning of exposure. Peak excretion of 2,4-TDA occurred 12 hours after the beginning of exposure. The half-lives for the excretion of 2,4-TDA and 2,6-TDA were 20.1 and 22.7 hours, respectively. Cumulative levels of excretion (sum of both metabolites) of 5.5, 32.5 and 155 µg (corresponding to 45, 266 and 1269 nmol (with a TDA molar mass of 122.17 g/mol))

were derived for TDA doses of 0.2%, 1% and 5% from diagrams that mapped TDA elimination over time. Accordingly, 0.29% to 0.34% of the amount of TDI applied to the skin was excreted as TDA.

3.2 Metabolism

The metabolism was described in detail in the 1999 supplement (Greim 1999). TDI preferably forms adducts with NH_2, OH and SH groups of proteins. The isocyanates first undergo hydrolysis, forming the corresponding amines or carbamic acid esters; these, in turn, react with residual isocyanate (and moisture) to form complex polyurea mixtures following a sequence of additional reaction steps. This type of polymerized, in most cases precipitated material of a high molar mass is not bioavailable, but is excreted by mucociliary clearance via the gastrointestinal tract. The formation of TDA depends on the pH. A small amount of TDA is formed in the lungs at a pH of 7. In the stomach, a comparatively much larger amount of TDA is formed because of the low pH of about 2.

4 Effects in Humans

4.1 Single exposures

The volunteer studies for odour perception, irritation and lung function after TDI exposure are shown in detail in Table 2.

No odour was perceived by 5 to 6 test persons who were exposed for 30 minutes to 10 or 20 µl/m³ of a mixture of 2,4-TDI and 2,6-TDI (65% : 35%) or to 20 µl/m³ of 2,4-TDI or 2,6-TDI as individual substances. At concentrations of 50 µl/m³ and above, all volunteers perceived the odour and 5 volunteers reported an initial "tingling" sensation on the conjunctiva and "stinging" in the nose with slight discharge. The intensity of the perceived odour and irritation were more marked after exposure to a 2,6-TDI concentration of 50 µl/m³ than after a 2,4-TDI concentration of 50 µl/m³. At 75 µl/m³ and above, the symptoms of irritation of the eyes and nose were more severe. The colorimetric determination of the TDI concentrations in the exhaled air was described in detail (Henschler et al. 1962). These symptoms and defence mechanisms suggest sensory irritation (Brüning et al. 2014).

A 20-minute bronchial inhalation challenge test with a TDI concentration of 20 µl/m³ (no other details) and a methacholine provocation test were carried out on different days in a total of 40 volunteers. Twenty of the volunteers were exposed to TDI while working in a foam factory (exposed persons) and reported occasional symptoms of asthma. The tests were carried out on symptom-free days. The control group was made up of 20 volunteers who were not exposed; 10 of them were healthy with a normal lung function (healthy persons) and 10 had extrinsic asthma symptoms (asthmatics). A positive result was defined as a greater than 50% increase in the specific airway resistance (sRaw) compared with the initial value during the 6-hour observation period following exposure to TDI. The sRaw increased by more than 50% in 9 of the 20 persons exposed to TDI. Effects on the sRaw were not found in any of the asthmatics or healthy persons (Chester et al. 1979).

None of a group of 17 healthy volunteers (8 smokers and 9 non-smokers) reported irritant effects after 6-hour exposure to a 2,4-TDI/2,6-TDI mixture in a concentration of 5 µl/m³ followed by 20-minute exposure to a 2,4-TDI/2,6-TDI mixture in a concentration of 20 µl/m³. The total cell count in the bronchial lavage fluid (BLF) or in the BALF was not affected. Albumin was increased in the BALF ($p = 0.044$; 26.4 compared with 21.8 µg/ml) and α_2-macroglobulin was increased in the BLF ($p = 0.021$; 0.07 compared with 0.05 µg/ml) (overlapping confidence intervals). Lung function tests yielded a slight increase in the sRaw and a decrease in the maximum expiratory flow at 25% of the forced vital capacity. No differences were found in the effects induced in smokers and non-smokers (Vandenplas et al. 1999).

Tab. 2 Volunteer studies with TDI exposure

Concentration (µl/m³)	Composition	Test persons	Exposure period	Findings	References
20	no data	3 healthy persons, sex not specified	1 to 5 minutes	not noticeable	Bayer AG 1970
10, 20	2,4-TDI/2,6-TDI mixture (65% : 35%)	6 healthy men	30 minutes	no perception of odour	Henschler et al. 1962
50			30 minutes	6/6: perception of odour and adaptation to odour, 5/6: tingling sensation on the conjunctiva 1/6: stinging in the nose with slight discharge 1/6: no unusual findings	
75			30 minutes	6/6: odour more marked, mild burning sensation, no lacrimation, tingling sensation or mild stinging in the nose when breathing in	
100			30 minutes	6/6: symptoms of the eyes and nose more severe, mild lacrimation, 2/6: slight irritation of the throat	
500			30 minutes	6/6: lacrimation, burning sensation and tickling in the throat	
1300	2,4-TDI/2,6-TDI mixture (65% : 35%)	2 healthy men	10 minutes	severe lacrimation, tightened lids, severe irritation of the conjunctivae; several hours later: catarrhal signs in the airways with coughing	
20	2,4-TDI	5 healthy men	30 minutes	no perception of odour	
50			30 minutes	mild odour perception, no irritation	
80–100			30 minutes	increase in conjunctival irritation and tingling in the nose	
200–500			30 minutes	200 µl/m³: 2/5: burning eye irritation, annoying; 500 µl/m³: 5/5: burning eye irritation, annoying	
20	2,6-TDI	5 healthy men	30 minutes	no perception of odour	
50			30 minutes	perceived odour and eye irritation much more severe than after exposure to 2,4-TDI	
80–200			30 minutes	more severe symptoms of irritation of the eyes and nose, feeling of dryness in the throat, no tickling sensation	
500			30 minutes	5/5: burning eye irritation, annoying, no differences in the effects of the isomers	
20	TDI (no other details)	10 healthy persons, 10 asthmatics (no other details)	20 minutes	NOAEC for sRaw (increase of > 50%)	Chester et al. 1979
10	TDI (no other details)	10 healthy persons (no other details)	60 minutes	no asthmatic reaction (criterion: 100% increase of airway resistance amounting to 0.5 kPa/l per second or more)	Baur et al. 1994
5, 20	2,4-TDI/2,6-TDI mixture (80% : 20%)	17 healthy persons; of these 6 male smokers, 2 female smokers, 2 male non-smokers, 7 female non-smokers, 35 years (19–51 years)	5 µl/m³: 6 hours, (directly afterwards) 20 µl/m³: 20 minutes, 2 exposures at 4-week intervals	no differences between smokers and non-smokers **lung function:** slight increase in sRaw ($p = 0.053$) slight decrease in $MEF_{25\%}$ ($p = 0.015$) **BLF/BALF:** no effects on the total cell count, albumin (BALF)↑ ($p = 0.044$), [∅ 26.4 compared with 21.8 µg/ml], α_2-macroglobulin (BLF)↑ ($p = 0.021$), [∅ 0.07 compared with 0.05 µg/ml] (overlapping CI)	Vandenplas et al. 1999

BALF: bronchoalveolar lavage fluid; BLF: bronchial lavage fluid; CI: confidence interval; $MEF_{25\%}$: maximum expiratory flow at 25% of the forced vital capacity; sRaw: specific airway resistance

Summary: Irritation, primarily in the form of sensory irritation, was induced by exposure to a 2,4-TDI/2,6-TDI mixture in concentrations of 50 µl/m³ and above and by exposure to the individual isomers (Henschler et al. 1962). Lung function parameters such as sRaw and the maximum expiratory flow at 25% of the forced vital capacity were slightly decreased after 6-hour exposure to a 2,4-TDI/2,6-TDI mixture in a concentration of 5 µl/m³ followed by 20-minute exposure to a 2,4-TDI/2,6-TDI mixture of 20 µl/m³. Albumin in the BALF and α_2-macroglobulin in the BLF were increased. As two different concentrations were used in succession, the effects cannot be attributed with certainty to a specific concentration. The effects observed after exposure to a 2,4-TDI/2,6-TDI mixture of 5 µl/m³ are regarded as slight. A NOAEC (no observed adverse effect concentration) of about 5 µl/m³ was derived from the volunteer studies for irritation caused by a 2,4-TDI/2,6-TDI mixture; the NOAEC for changes in lung function induced by a 2,4-TDI/2,6-TDI mixture is also about 5 µl/m³ (Vandenplas et al. 1999).

4.2 Repeated exposure

A large number of studies that investigated repeated exposure at the workplace are available; the studies carried out until 1998 were described in detail in the 1999 supplement (Greim 1999). All workplace studies that were published after 1998 and the earlier studies that are relevant for the derivation of a MAK value are shown in Table 3 of this supplement. The studies published after 1998 are described in detail below.

4.2.1 TDI production

In the period from 2007 to 2012, a longitudinal study was carried out in 197 of a total of about 300 workers at 3 TDI production plants. The levels of exposure (TWA (time-weighted average) and peak concentrations) were determined in about one third of the workers every 3 months by means of personal air sampling. The workers were classified in 10 exposure groups depending on their work activities. A total of 1594 TWAs and 755 peak concentration values were available for evaluation. The 2,4-TDI and 2,6-TDI concentrations were determined separately and used to calculate the total amount of TDI. Stationary sampling or biomonitoring was not carried out. The arithmetic mean of the TWAs over the total period and all work areas was 0.65 µl/m³ (0.01–92 µl/m³); concentrations between 2 and < 5 µl/m³ were determined in 49 cases (3%) and levels ≥ 5 µl/m³ (up to 92 µl/m³) were determined in 35 cases (2.2%). Peak concentrations of up to 1726 µl/m³ were recorded at workplaces using respiratory protection and peak concentrations of up to 347 µl/m³ were recorded at laboratory workplaces without respiratory protection. The workers were examined each year; the examinations included spirometry and the documentation of medical histories and spontaneously occurring symptoms. Of the 179 workers who completed continuous questionnaires, 118 reported that they perceived the odour of TDI. Information about dermal exposure was not obtained. However, dermal exposure of the workers was assumed to have occurred only sporadically (Middendorf et al. 2017). Seven workers were diagnosed with occupational asthma that was probably caused by TDI, 2 workers with probable occupational asthma with unclear genesis and 23 workers were found to have symptoms of asthma with unclear genesis. In the cases with TDI-induced asthma, the odds ratios (OR) were increased for cumulative exposure (OR = 2.08 (95% CI: 1.07–4.05)) and for exposure to peak concentrations (OR = 1.18 (95% CI: 1.06–1.32)). In all 23 cases with asthma of unclear genesis, the ORs for cumulative exposure (OR = 1.2; 95% CI: 0.87–1.66) or for exposure to peak concentrations (OR = 1.04; 95% CI: 0.99–1.11) were not increased. All 7 workers with TDI-induced asthma reported having (repeatedly) perceived the odour of TDI or having been in an area where TDI was released (Collins et al. 2017). An annual decrease in the forced expiratory volume (FEV_1) of more than 350 ml/year or at least 10% was observed in the lung function tests of 19 workers; 17 of these workers had no asthma symptoms. On average, the FEV_1 and forced vital capacity (FVC) values of the total male cohort were below the expected levels both in the initial examination and in the final examination (%RefFEV_1 93.7/92.4 and %RefFVC 92.5/91.3). The findings were similar when the 127 non-smokers were evaluated separately (27/35 abnormal lung function tests, 24/32 with a restrictive pattern). The findings tended to correlate with cumulative exposure. The cases with asthma or effects on the lung function were not categorized by previous exposures (Collins et al. 2017; Middendorf et al. 2017; Wang et al. 2017).

A longitudinal study covering the period from 1967 to 1992 collected data for 313 workers and 158 control persons from the medical records of the occupational clinic and self-initiated visits to the clinic attributed to incidents of exposure.

From 1980 onwards, standardized questionnaires and spirometry were used for the evaluation. The mean duration of employment was 4.7 to 5.7 years. Exposure was determined by stationary sampling until 1976 and by personal sampling until the end of the study. There are no data available for skin contact. The average TWA related to the total period and all areas of work was 4.2 µl/m³ and the mean cumulative exposure for 267 workers was 234.2 µl/m³ × month. The concentrations of both 2,4-TDI and 2,6-TDI were determined and given as the sum. No relationship was found between cumulative exposure and the effects on lung function (FVC and FEV_1). No effects on the annual decrease in FEV_1 or FVC were observed in the 209 men with exposure in comparison with 65 control persons. TDI may have induced asthma in 19 cases (13 × symptoms, 3 × symptoms and medical evaluation, 3 × symptoms and lung function test) (Ott et al. 2000).

In the period from 1971 to 1997, 305 workers who were employed in a plant for at least 3 consecutive months and 581 control persons underwent medical examinations every 1 to 2 years. A total of 449 personal sampling results from 20 work areas were available. The average TWAs, calculated as the sum of 2,4-TDI and 2,6-TDI, were 2–12 µl/m³ (1971 to 1979) and 1–3 µl/m³ (1979 to 1980) and the average cumulative exposure was 96.9 µl/m³ × month (maximum of 639 µl/m³ × month). Exposure peaks were not determined and data for skin contact were not available. Effects on the lung function were not observed. Among the symptoms that were specifically asked about, asthma and coughing were reported more frequently in the course of exposure than at the beginning (initially 2.9%⟶6.4%); however, these symptoms were reported also by control persons (initially 5.9%⟶9.2%). Persistent coughing was the only symptom that was reported more frequently by the exposed persons (20.2%) than by the control persons (19.3%). Adjustment was made for smoking habits, ethnic group, sex, size, age, time of first exposure, initial prevalence of respiratory symptoms and baseline lung function values. Irritation was not reported (Bodner et al. 2001).

4.2.2 Companies producing polyurethane foam (PU production)

In a longitudinal study (Clark et al. 1998; see also Greim 1999) covering the period from 1981 to 1986, lung function parameters were determined in 780 workers who were employed in 12 plants in the United Kingdom for up to 5 years. The workers were questioned about symptoms and asthma (the results of 217 workers were additionally included in the follow-up study of Clark et al. (2003)). In the United Kingdom, the maximum 8-hour mean exposure limit for isocyanates, expressed as isocyanate group (NCO), is 0.02 mg/m³; this corresponds to an average TDI concentration of 5.8 µl/m³ and a cumulative TDI level of 46 µl/m³ × hour for an 8-hour working day. The 15-minute peak limitation value valid for NCO in the United Kingdom is 0.07 mg/m³; this corresponds to a TDI concentration of 20 µl/m³. A total of 2294 personal TDI samples were obtained; 4.7% of the determined levels exceeded the exposure limit of 46 µl/m³ × hour. During the study period, the exposure limit was exceeded daily by 5 of 780 persons (= 0.6%), and exposure was below 10 µl/m³ × hour for 79.1% of the persons. The peak concentrations were above 40 µl/m³ and 20 µl/m³ in 8.8% and 19% of the samples taken, respectively. The exposed persons were classified according to their activities in 3 exposure groups: a low exposure group (97 male and 39 female persons) with exposure up to 10 µl/m³ × hour, a handling group (80 male and 43 female persons) with levels up to 20 µl/m³ × hour and a high exposure group (472 male and 49 female persons) with levels up to 80 µl/m³ × hour and above. No information is available whether the 2,4-TDI and 2,6-TDI levels were analysed separately or in total. Shortness of breath and wheezing were reported more frequently in the handling and high exposure groups at the end of exposure compared with the beginning of exposure; the increased frequency was statistically significant. Likewise, the incidences of shortness of breath, wheezing and chest pain were higher among the workers of these 2 groups who had left the plants than among those who continued to be employed there. The incidence of symptoms was not increased with statistical significance in the group of workers who were exposed to levels that were, on average, below 10 µl/m³ × hour a day. During the study, 24 cases of respiratory sensitization were diagnosed. These cases were not assigned to any of the exposure groups. Effects on the lung function were not observed in the different exposure groups (FEV_1, FVC and peak expiratory flow). The correlation between the decrease in the FEV_1 and the age of the workers to the square ($p < 0.05$) and the smoking habits ($p < 0.1$) was statistically significant, as was the correlation between the decrease in the FVC and age ($p < 0.01$) and age to the square ($p < 0.05$). Changes in the peak expiratory flow correlated with the age of the workers, past exposure before the beginning of the study and the recorded peak concentrations. In a subgroup of naive exposed workers who joined the study 1 year later, the annual decreases in the FEV_1 and FVC correlated with the mean daily levels of exposure compared with the

values determined in non-naive exposed persons. An evaluation of the total study population found that the average decrease in the FEV_1 of 40 ml/year in the workers who had left the plant did not differ with statistical significance from the FEV_1 of 38 ml/year in the workers who continued to be employed there. Among the workers who had left the plant, statistically significant increases in wheezing, shortness of breath and chest pain and statistically significant decreases in the FEV_1 (71 ml/year) were reported only in the group exposed up to 20 µl/m³ × hour. The authors concluded that this study did not show that the FEV_1, FVC or peak expiratory flow were decreased in workers exposed at levels below the valid mean 8-hour exposure limit of 5.8 µl/m³ (Clark et al. 1998). Irritation or skin contact was not reported. A NOAEC of 1.25 µl/m³ (10 µl/m³ × hour: 8 hours) has been derived because a comparison of the findings in the low exposure group after exposure at levels below 10 µl/m³ × hour and the data obtained at the beginning of the study did not yield any changes in the lung function parameters FEV_1, FVC or peak expiratory flow and the symptoms such as shortness of breath, wheezing and chest pain did not occur more frequently.

A longitudinal follow-up study covering a period of 2 years from 1997 to 1998 re-examined and questioned 251 workers, 217 of them from the study of Clark et al. (1998). Of the participants, 229 had been continuously employed at the plants from 1986 to 1998. In 1998, 70 of the 251 workers were re-classified into other exposure groups. No information is available whether the 2,4-TDI and 2,6-TDI levels were analysed separately or in total. The data for 8-hour exposure to TDI obtained from 1004 personal samples showed that 89.6% were exposed to levels up to a maximum of 20 µl/m³ × hour, 9.1% to between 20 and 40 µl/m³ × hour and 1.3% to above 40 µl/m³ × hour. None of the 251 workers was exposed to TDI concentrations that exceeded 5.8 µl/m³ a day. 230 (92%) of the workers were found to be exposed to TDI concentrations of less than 15 µl/m³ × hour (1.9 µl TDI/m³). About 123 were exposed to levels of up to 5 µl/m³ × hour, 65 to between 5 and 10 µl/m³ × hour, 42 to between 10 and 15 µl/m³ × hour, and the remaining workers were exposed to levels between 21 and 30 µl/m³ × hour (data taken from a graph). The workers were classified into 3 exposure groups: 1.05 µl/m³ (production workers; n = 175); 0.6 µl/m³ (workers who handled the hardened foam; n = 26) and 0.3 µl/m³ (workers not involved in production; n = 50 control persons). The range of exposure was not reported for each specific group; however, exposure during production probably reached levels up to 30 µl/m³ × hour (3.75 µl/m³). Effects on the lung function were not induced by the exposure to TDI. The annual decreases in the FEV_1 and FVC of 35 ml and 30 ml, respectively, were about the same as those determined in the period from 1981 to 1986 and thus in the range of those of workers not exposed. The annual decreases in the FEV_1 and FVC correlated with the increases in weight, smoking habits and age. The 41 naive workers included in Clark et al. (1998) were found to have an annual decrease in the FEV_1 of 39 ml/year compared with that of 34.5 ml/year in the non-naive workers. However, this decrease correlated only with ex-smoker status, but not with exposure. The percentage frequency of the most commonly reported symptoms was higher at the beginning of the study than at the end. The percentage of workers in production (average of 1.05 µl/m³) who reported shortness of breath and wheezing was increased; however, the percentage of control persons who reported wheezing was also higher. Likewise, the percentage of workers with shortness of breath was increased in the handling group. The data did not provide evidence of a correlation of the symptoms with exposure or the exposure period. Irritation was not reported. There are no data available for skin contact (Clark et al. 2003).

A total of 136 workers from 11 plants producing polyurethane foam were questioned about effects (coughing, coughing with mucus, breathing noises and symptoms of the eyes and nose) they had possibly experienced within the last 12 months. These data were compared with those of 118 control persons. Adjustment was made for smoking habits, sex and age. The workers were on average exposed to 2,4-TDI concentrations of up to 2.6 µl/m³ and to 2,6-TDI concentrations of 0.01 to 3.6 µl/m³ (personal samples). Concentrations of 0.004 to 5.2 µl/m³ were reported for the sum of the 2 isomers (personal samples; 8 hours). The workers were additionally exposed to other substances: in 4 plants to low or not detectable concentrations of MDI (means: 0.03 and 0.35 µl/m³; range: 0.004–0.75 µl/m³), in 1 plant to isophorone diisocyanate (means: 0.03 µl/m³; range: 0.01–0.1 µl/m³) and in 2 plants to 1,5-naphthalene diisocyanate (means: 0.22 and 0.49 µl/m³; range: 0.17–1.8 µl/m³) and phenyl isocyanate (means: 0.05 and 0.2 µl/m³; range: 0.02–0.33 µl/m³) (Sennbro et al. 2004 a). The percentage of asthma and bronchitis cases was lower in the group of exposed workers than in the control persons. Symptoms of the eyes, coughing, coughing with mucus, attacks of dry coughing and nose bleeds were reported more frequently by the exposed workers; the increased frequency was statistically significant. The symptoms were not assigned to exposure concentrations or the duration of exposure. 2,4-TDA concentrations of up

to 623 nmol/l (limit of detection: 0.41 nmol/l) and 2,6-TDA concentrations of up to 353 nmol/l were detected in the urine; 2,4-TDA concentrations of up to 254 nmol/l and 2,6-TDA concentrations of up to 509 nmol/l were determined in the plasma. The authors reported that the TDI concentrations obtained by personal sampling correlated well with the TDA concentrations in the urine and plasma (Littorin et al. 2007). In a group of 81 workers from these plants, the external exposure concentrations determined by personal sampling (concentrations up to 6.1 µl/m³; sum of 2,4-TDI and 2,6-TDI) correlated with the TDA levels in the plasma and urine. The TDA values determined in the urine were between 0.1 and 162 µg/l, and there was a linear correlation between the external exposure and body burden (Sennbro et al. 2004 b). The study of Littorin et al. (2007) suggests that irritation of the eyes and upper airways is expected to occur at TDI concentrations up to 6.3 µl/m³. A no-effect concentration was not derived from these results. However, plants that produce polyurethane foam use a large number of substances such as solvents, and polyurethane dust may develop during production. Therefore, the effects cannot explicitly be attributed to the exposure to the 2,4-TDI/2,6-TDI mixture. Data for skin contact were not reported.

In a 1-year follow-up study in a newly opened polyurethane foam plant (study dates not specified), 49 workers were exposed to maximum TDI concentrations of 10 µl/m³ (foaming area) and 5.4 µl/m³ (cutting area). 2,4-TDI and 2,6-TDI levels were not determined separately. Of the 49 workers, 42 and 37 workers were still available after 6 and 12 months of observation, respectively. TDI was sampled stationary. About 90% of these samples were below the limit of detection for TDI of 0.1 µl/m³. Likewise, the results obtained from a "limited number" of personal samples from 7 workers were below the limit of detection (no other details). Thirteen workers reported in the questionnaire that they were additionally exposed to TDI via the skin. In the group of 49 workers, 3 had asthma symptoms. After 6 months, 3 of the remaining 42 workers reported "new asthma symptoms". After 12 months, none of the remaining 37 workers reported "new asthma symptoms". Asthma symptoms were reported by 3 of the 12 workers who were no longer available after 12 months; therefore, a much higher fraction was found in these workers than in the overall group of 49 workers. TDI-specific IgE tests carried out after 12 months yielded negative results for all 37 workers. The TDI IgG serum level was increased in 1 exposed worker after 6 months. The effects were no longer observed 12 months after this worker was transferred to an area with lower exposure. After 1 year, the FEV_1 was decreased by more than 15% in 3 of 33 workers and 1 worker had pulmonary obstruction (4 of the 37 remaining workers were not monitored). Eye irritation was reported by 1 of 42 workers and 1 of 37 workers after 6 and 12 months, respectively (Gui et al. 2014).

Tab. 3 Studies after long-term exposure to TDI at the workplace

Cohort, exposure duration, (study period)	Exposure level	Exposure peaks	Findings	References
TDI production				
305 workers, at least for 3 consecutive months, 581 control persons, no data, (1971–1997)	449 personal samples: TWA: 2–12 µl/m³ (1971–1979); 1–3 µl/m³ (1979–1980); cumulative exposure: 96.9 µl/m³ × months (maximum: 639 µl/m³ × month)	no data	**lung function**: no effects **asthma**: asthma/coughing (initially 2.9%⟶6.4%; control persons: 5.9%⟶9.2%) **irritation**: no data	Bodner et al. 2001
3 plants, 197 of 300 workers, 15.7 years, (2007–2012)	total period: TWA: 0.65 µl/m³ 2– < 5 µl/m³ (n = 49; 2.8%); ≥ 5 µl/m³ (up to 92 µl/m³) (n = 35; 2.2%); up to 12.4 µl/m³ (laboratory), up to 32.7 µl/m³ (loading) only a few determinations (maximum of 6) taking into account respiratory protection	up to 1726 µl/m³ (with respiratory protection), laboratory work: up to 347 µl/m³ without respiratory protection, STEL (20 µl/m³) exceeded in 5/121 determinations in a laboratory	**lung function**: 19 times at least one 1-year decrease in the FEV_1 > 350 ml or at least 10%, of these 17 times without asthma symptoms, 27/35 abnormal lung function, restrictive pattern in 24/32 of these **asthma**: 7 cases of TDI-induced asthma (TDI odour perceived) **irritation**: no data	Collins et al. 2017; Middendorf et al. 2017; Wang et al. 2017

Tab. 3 *(continued)*

Cohort, exposure duration, (study period)	Exposure level	Exposure peaks	Findings	References
200 exposed workers, 54 control persons, 5 years, (1973–1978)	0.1–25 µl/m³; low: TWA 1.6 µl/m³ (n = 659 determinations; 2.1% > STEL (20 µl/m³)); medium: TWA 3.2 µl/m³ (n = 851; 16.6% > STEL); high: TWA 6.8 µl/m³ (n = 439; 44.2% > STEL); cumulative exposure: group 1: < 68.2 µl/m³ × month, group 2: ≥ 68.2 µl/m³ × month	peak exposure: > 20 µl/m³ over a maximum period of 0.19 months (5 days); > 20 µl/m³ over a period of more than 0.19 months (5 days); > STEL about 6.2% of working hours	**lung function**: non-smokers: cumulative duration of peak exposure: average annual decrease in the FEV_1 higher in the medium exposure group than in the low group (–30 ml compared with –6 ml); cumulative exposure: average annual decrease in the FEV_1 in the medium group higher than in the low group (–37 ml compared with +1 ml) **asthma**: suspected sensitization to TDI in 12 workers: 6 workers with known high exposure; provocation test with 20 µl/m³ positive in 2 of 6 **irritation**: no data	Diem et al. 1982
313 workers, 158 control persons, women: 4.7 years, men: 5.7 years; 3 months–30 years, (1967–1992)	total period: 4.4 µl/m³, cumulative: 234.2 ml/m³ × month	no data	**lung function**: no effects **asthma**: 19 cases **irritation**: no data	Ott et al. 2000
PU production				
251 workers (217 of these from the study of Clark et al. (1998), 2 years, (1997–1998)	1.05 µl/m³ (A: workers in production; 175); 0.6 µl/m³ (B: handling of the hardened foam; 26); 0.3 µl/m³ (C: non-production workers; 50 = control persons)	1004 personal samples: 1–5 µl/m³ × hour (n = 263); 5–10 µl/m³ × hour (n = 418); 10–20 µl/m³ × hour (n = 219); 20–40 µl/m³ × hour, (corresponds to 5.8 µl/m³) (n = 91); > 40 µl/m³ × hour (n = 13); 230 workers (92%): < 15 µl/m³ × hour (corresponds to 2 µl/m³)	**lung function**: no effects induced by TDI (annual decrease in the FEV_1 of 35 ml or decrease in the FVC of 30 ml); shortness of breath ↑ in group B (2/26⟶5/26), wheezing ↑ in group A (22.9%⟶32.6%; mainly in smokers; 9.7%⟶15.3% in 72 non-smokers) **asthma**: not examined (sensitized persons were removed from the area of exposure) **irritation**: no data	Clark et al. 2003
49 workers, 1 year (41% occasional smokers, 16% ex-smokers, 43% non-smokers) (12 dropped out after 12 months)	≈ 90% < 0.1 µl/m³ (LOD); values of "limited" personal sample results in 7 workers likewise < LOD (no other details)	maximum: 5.4 (cutting area) up to 10 µl/m³ (foaming area)	**lung function**: decrease in the FEV_1 of > 15% in 3/33 after 1 year (no determination in 4 of the 37 remaining workers) **asthma**: beginning: 3/49; after 6 months: 3/42 "new asthma symptoms", 3/12 drop-outs **irritation**: eye irritation 1/42 (6 months), 1/37 (12 months)	Gui et al. 2014
2 plants, 386 exposed workers, initial lung function available for 294, at least 2 years, (1982–1987)	average TWA 2 µl/m³ (1.2–4.5 µl/m³)	peaks (12 minutes) above 5 µl/m³ in 9% of the determinations and above 20 µl/m³ in 1% of the determinations	**lung function**: no acceleration of the age-dependent decline in lung function when asthma cases were excluded, increase in chronic bronchitis with an increase in cumulative exposure, a number of lung function parameters below expected value after high cumulative exposure **asthma**: 12 cases of sensitization (half with a positive result in the provocation test) **irritation**: no data	Jones et al. 1992

Tab. 3 *(continued)*

Cohort, exposure duration, (study period)	Exposure level	Exposure peaks	Findings	References
26 men, 26 control persons, 6.6 years, (no data)	35–370 µl/m³ (30–90 minutes)	no data	**lung function**: before the shift: exposed workers: FEV_1/FVC: 83%, control persons: FEV_1/FVC: 89.3% **asthma**: negative results for TDI in provocation tests **irritation**: eye irritation 13/26 (control persons 2/26), coughing 13/26 (control persons 2/26), rhinitis 9/26 (control persons 5/26) no time-dependency if divided into groups with < 10 years and ≥ 10 years of exposure	Lee and Phoon 1992
136 exposed workers (38 atopics, 13 bronchitis, 89 smokers/ex-smokers), 118 control persons (33 atopics, 83 smokers/ex-smokers), no data, (2000–2001)	8-hour mean, personal samples, 79 exposed workers: up to 2.6 µl/m³ (2,4-TDI), 0.01–3.6 µl/m³ (2,6-TDI), low to not detectable exposure to MDI, IPDI, NDI, PI	up to 28–38 µl/m³	**lung function**: not examined **asthma**: 12/138 exposed workers, 13/118 control persons **irritation**: symptoms of the eyes↑ (49/136, control persons: 5/118), wheezing↑ (33/118, control persons: 2/118), coughing (38/136, control persons: 19/118), dry coughing (28/136, control persons: 3/118), nose bleeds (15/136, control persons: 1/118)	Littorin et al. 2007; Sennbro et al. 2004 a, b
3 plants, 57 exposed workers, 24 control persons, 17 years, (1983–1985)	average TWA: 5.7 µl/m³ (29 workers with high exposure, HE); 0.1 µl/m³ (peaks: < 1 µl/m³; 28 workers with low exposure, LE)	classification of HE: HE1: n = 14; TWA: Ø 1.7 µl/m³ (maximum 4 µl/m³; peaks: 3–14 µl/m³); HE2: n = 15; TWA: Ø 8.2 µl/m³ (maximum 30 µl/m³; peaks: 30–80 µl/m³)	**lung function**: acceleration of the age-dependent decline (%FEV_1 [–0.81 compared with –0.28], %MMF [–2.39 compared with +1.91], %MEF25 [–3.28 compared with +3.38], %MEF50 [–2.42 compared with +1.27], %PEF [–0.38 compared with +0.66] in HE2 greater than in HE1 (not %FVC [+0.1 compared with –0.49])). No differences: HE1 and control persons; LE and control persons **asthma**: no data **irritation**: no data	Omae et al. 1992
37 exposed workers, 67–82.5 months, (1972–1976)	in 111–138 personal samples (20–90 minutes; always on the days of lung function monitoring and periodically during the first 2 years): 0.5–13 µl/m³; group A: < 2 µl/m³ (0–4 µl/m³, 10 ♂, 3 ♀); group B: 2–3.4 µl/m³ (0–14 µl/m³, 6 ♂, 7 ♀); group C: > 3.5 µl/m³ (0–40 µl/m³, 11 ♂)	no data	**lung function**: significant decrease in the FEV_1 over the shift correlated with the level of exposure independent of age and smoking habits, after 4 years: significant increase in the average annual decline in the FEV_1 in group C (standardized 65 ml compared with 0 ml in group A), higher decrease mainly in the first 2 years of the study **asthma**: no data **irritation**: no difference in the frequency of symptoms of the upper/lower airways	Wegman et al. 1982

FEV_1: forced expiratory volume; FVC: forced vital capacity; IPDI: isophorone diisocyanate; LOD: limit of detection; MDI: diphenylmethane-4,4′-diisocyanate; %MEF: maximum expiratory flow at 25%/50% of the forced vital capacity; %MMF: maximum mid-expiratory flow; NDI: 1,5-naphthylene diisocyanate; %PEF: peak expiratory flow; PI: phenyl isocyanate; STEL: short-term exposure limit; TWA: time-weighted average

Tab. 4 Summary of the NOAECs/LOAECs for various end points in humans

Ø TWA (µl/m³)		Peak exposures	End point or effects	References
NOAEC	**LOAEC**			
lung function				
0.9	1.9	6% of the working hours > 20 µl/m³	annual decrease in the FEV_1	Diem et al. 1982
1.05	no information possible	up to 2.5 µl/m³ (20 µl/m³ × hour) 90% of the determinations	symptoms (wheezing, mainly in smokers)	Clark et al. 2003
< 1.25	> 10 µl/m³ × hour	19% of the determinations > 20 µl/m³	annual decrease in the FEV_1 in naive workers	Clark et al. 1998
1.7	8.2	up to 14 (at the NOAEC) / up to 80 µl/m³	decrease in %FEV_1, %MMF, %MEF_{25}, %MEF_{50} and %PEF	Omae et al. 1992
2	> 2	up to 14 (at the NOAEC) / up to 40 µl/m³	annual decrease in the FEV_1	Wegman et al. 1982
2	no information possible	9% of the determinations > 5 µl/m³ and 1% > 20 µl/m³	annual decrease in the FEV_1 after excluding the asthma cases	Jones et al. 1992
2.3	no information possible	no determinations	annual decrease in the FEV_1	Bodner et al. 2001
4.4	no information possible	0.9 × and 0.5 × per shift > 20 µl/m³	annual decrease in the FEV_1 and FVC	Ott et al. 2000
no information possible	no information possible	stationary sampling up to 10 µl/m³	symptoms, lung function (annual decrease in the FEV_1)	Gui et al. 2014
irritation				
0.1 (LOD, 85%–95% of all values)	no information possible	stationary sampling up to 10 µl/m³	eye irritation: 6 months: 1/42; 12 months: 1/37	Gui et al. 2014
< 5 (6 hours)	no information possible	volunteer study	no irritation described	Vandenplas et al. 1999
up to 20 (30 minutes)	50 (30 minutes)	volunteer study	no odour perception	Henschler et al. 1962

FEV_1: forced expiratory volume; FVC: forced vital capacity; LOD: limit of detection; %MMF: maximum mid-expiratory flow; %MEF: maximum expiratory flow at 25%/50% of the forced vital capacity; %PEF: peak expiratory flow; TWA: time-weighted average

Summary: Data for irritation and effects on lung function are available from both volunteer and workplace studies. The volunteers did not perceive odour or report any irritation after exposure to 20 µl/m³ for 30 minutes (Henschler et al. 1962). After short-term exposure (30 minutes), initial signs of sensory irritation were observed at 50 µl/m³ (Henschler et al. 1962). In a workplace study (Gui et al. 2014), eye irritation was observed in 1 of 42 workers after 6 months and in 1 of 37 workers after 12 months; the irritation was probably caused by exposure peaks of 10 µl/m³. Therefore, it is assumed that irritation does not occur in humans at levels below 5 µl/m³. It was not possible to derive a definite NOAEC for the end point "lung function" from the volunteer studies because 6-hour exposure to a 2,4-TDI/2,6-TDI mixture at a concentration of 5 µl/m³ followed by 20-minute exposure to a 2,4-TDI/2,6-TDI mixture at a concentration of 20 µl/m³ resulted in a slight increase in the sRaw and a decrease in the maximum expiratory flow at 25% of the forced vital capacity ($MEF_{25\%}$) (Vandenplas et al. 1999). These effects were probably induced by the 20-minute exposure to the 2,4-TDI/2,6-TDI mixture at a concentration of 20 µl/m³. This assumption is supported by the workplace studies that no longer found changes in lung function parameters at TDI concentrations of about 1 to 2 µl/m³. Therefore, the data in humans demonstrate that exposure to TDI in a concentration range of 1 to 2 µl/m³ for 6 to 8 hours does not induce irritation or effects on the lung function. Acute effects in the form of sensory irritation were observed at TDI concentrations of about 50 µl/m³ and above. Nevertheless, it must be assumed that exposure peaks increase the severity of effects and should be avoided as far as possible. Animal studies (Devos et al. 2016) suggest that sensory irritation is based on the activation of the TRPA1 channel and neurogenic signal transduction pathways (release of substance P) may be involved in the development of asthma.

4.3 Allergenic effects

4.3.1 Sensitizing effects on the skin

The sensitizing effects of TDI on the skin were described in detail in the supplement from 2015 (Hartwig 2015). No new findings have been published since.

4.3.2 Sensitizing effects on the airways

4.3.2.1 Workplace studies

Since the publication of the supplement in 1999 (Greim 1999), a number of case reports of respiratory diseases presumably caused by TDI have become available that did not provide any details of the exposure conditions (for example Barbinova and Baur 2006; Raulf-Heimsoth et al. 2013; Scheidler et al. 2013; Sharifi et al. 2013). In addition, several cohort studies were published that investigated the effects of TDI at the workplace (Bodner et al. 2001; Clark et al. 2003; Collins et al. 2017; Gui et al. 2014; Meredith et al. 2000; Middendorf et al. 2017; NIOSH 2000; Ott et al. 2000; Wang et al. 2017) and included data for the frequency of developing respiratory diseases and exposure of the affected workers. It is not possible to derive a minimum induction concentration on the basis of either these studies or earlier studies (Clark et al. 1998; Diem et al. 1982; Jones et al. 1992; Omae et al. 1992; Wegman et al. 1974, 1977, 1982) that were included in the 1984 (Hartwig 2013) or 1999 (Greim 1999) documentation. Most of the studies mentioned did not include a clear diagnosis or provide evidence of sensitization; "asthma cases" were often (mainly) classified on the basis of symptoms reported by the workers themselves. Furthermore, the studies did not include sufficient data to attribute the symptoms to exposure. All of the available studies reported cases of asthma in workers defined as the "low dose group". Peak exposures were reported consistently, but these were likewise not assigned to specific exposed persons or exposure groups. Therefore, it is not clear at which level sensitization began to be observed. In addition, only in very rare cases was skin contact taken sufficiently into account. Therefore, on the basis of the available cohort studies, it is not possible to derive either a NOAEC with sufficient scientific reliability or a MAK value that provides protection from sensitization.

4.3.2.2 Provocation threshold in sensitized persons

In provocation tests lasting up to 50 minutes, 1 of 40 patients reacted to a TDI concentration of 5 µl/m^3 (no other details) (15-minute provocation), 3 persons reacted to 10 µl/m^3 (additional 30-minute provocation) and 8 patients reacted to 20 µl/m^3 (another 5-minute provocation). No skin reactions were observed in the remaining persons who were examined; however, longer periods of exposure were not investigated (Baur et al. 1994).

Eight patients with suspected occupational asthma induced by isocyanates underwent provocation testing with initial exposure to a TDI concentration of 5 µl/m^3 for 30 minutes followed by exposure to a TDI concentration of 10 µl/m^3 for 90 minutes (no other details); 1 patient produced a reaction. Methacholine tests were carried out prior to the provocation tests; these yielded findings of bronchial hyperreactivity in 3 of the 8 patients (Barbinova and Baur 2006).

Six persons with asthma suspected of having been induced by TDI underwent provocation testing with exposure to 4 increasing TDI concentrations (5, 10, 20 and 30 µl/m^3; no other details) each lasting 30 minutes. Reactions were observed in 2 of the 6 test persons (Raulf-Heimsoth et al. 2013).

Provocation with a TDI concentration of 1 µl/m^3 (no other details) for 45 minutes did not cause any notable decrease in the FEV_1 in 3 of 4 patients with suspected TDI asthma. However, the 4th patient produced a delayed reaction with a 23% decrease in the FEV_1 and slight increases in neutrophils and eosinophils in the BALF. A 3-minute provocation test carried out later in this patient and in 1 of the remaining 3 patients with a TDI concentration of 15 µl TDI/m^3 revealed slight increases in neutrophils and eosinophils, but no airway hyperreactivity (Lemière et al. 2002).

4.4 Genotoxicity

Since the publication of the 1999 supplement, 2 studies with workers who were exposed to a 2,4-TDI/2,6-TDI mixture were published for this end point.

Sister chromatid exchange (SCE), micronuclei and structural chromosomal aberrations were increased with statistical significance in the peripheral lymphocytes of workers exposed to 2,4-TDI/2,6-TDI (no other details) during plastics manufacturing at concentrations ranging from 7 to 16 µg/m³ (Bilban 2004). It is not possible to attribute the effects observed in this study to the 2,4-TDI/2,6-TDI mixture alone because no adjustment was made for the differences in age and smoking habits between exposed and control persons.

In a study, 42 workers with workplace-related airway symptoms (such as shortness of breath) were exposed for 2 hours to different diisocyanates (MDI, TDI or HDI) in a diagnostic provocation test that lasted 5 hours overall and was carried out after 5 days without exposure to diisocyanates. Ten workers were first exposed to a 2,4-TDI/2,6-TDI mixture (80% : 20%) at a concentration of 5 µl/m³ for 30 minutes, followed by exposure to 10 µl/m³ for 30 minutes. Then, after a 90-minute break, the workers were exposed to concentrations of 20 µl/m³ and 30 µl/m³, again for 30 minutes in each case. All participants in the study underwent medical examinations before testing, including lung function tests, methacholine testing and prick testing. Blood samples were analysed before exposure and 30 minutes and 19 hours after exposure. DNA double-strand breaks were determined in the lymphocytes immediately after blood sampling. Ten persons with mild asthma or bronchial hyperreactivity who were not occupationally exposed to diisocyanates were used as control persons; however, during the study itself, they were exposed to the different diisocyanates. During the exposure period, 17% of the 42 workers produced an asthmatic reaction, whereas no reaction was observed in the control group. On average, no increases in DNA double-strand breaks were observed in either the control persons or the workers at any time after exposure to the different diisocyanates (Marczynski et al. 2005).

4.5 Carcinogenicity

4.5.1 Cohort studies

Follow-up studies are available for the cohorts from England and Wales, Sweden and the United States; they reviewed mortality from tumours and cancer incidences (see Greim 1999).

A cohort study in England and Wales examined mortality (period from 1958 to 1998) and tumour morbidity (period from 1971 to 1994) in a total of 8288 women and men from 11 PU-producing plants who had worked there at least temporarily in the period from 1958 to 1979 and for at least 6 months overall. It was possible to update the work histories at the factories for 1195 of 1436 members of the cohort who were still employed at the end of 1986 (end of the original observation period). A total of 4612 factory/department/job combinations were defined on the basis of the exposure data that had been collected for the "MRC isocyanate study" from 1978 to 1986. An occupational hygienist classified 189 of these combinations as jobs with "higher" exposure (8-hour means for TDI above 4 µl/m³ or exposure peaks at TDI levels above 10 µl/m³ on most days), 701 as jobs with "lower" exposure (8-hour means for TDI of 1.5 to 4 µl/m³ or exposure peaks occasionally above a TDI level of 5 µl/m³) and 3628 as jobs involving "minimal or zero exposure". Compared with the cases determined in the general population, mortality from lung cancer was increased among women (35 observed cases; 19 expected cases; standardized mortality ratio (SMR): 181), and mortality overall and mortality from leukaemia and non-malignant diseases of the respiratory system were increased among men. For lung cancer, no clear trends were found for years since the beginning of work (stratified according to sex) or for years of employment with "higher" or "lower" exposure (adjusted for sex). No deaths from lung cancer were observed in the small group of 90 women with exposure (0.49 expected). Only about 1% of the members of the cohort were exposed to "higher" levels for longer than 5 years and only about 5% were exposed to "lower" levels for longer than 5 years. No information about dermal exposure was recorded. Data for changes in the exposure concentration during the observation period or for smoking habits were not available (Sorahan and Nichols 2002).

Another cohort study covering the period from 1959 to 1998 examined the mortality and cancer incidence in a total of 4175 women and men who were employed in 9 Swedish PU-producing plants for at least 1 year between 1959 and 1987. From 1965 onwards, the average concentrations of TDI were below 100 µg/m³; in the late 1980s, they were below 20 µg/m³. The MDI concentrations were lower than 10 µg/m³. Exposure peaks of up to 3000 µg/m³ were determined for TDI and up to 350 µg/m³ for MDI. The exposure concentrations decreased steadily over the years. An extensive analysis of the concentrations was carried out in 2000 and 2001. At 13 plants, the total isocyanate concentrations were determined to be, on average, 0.01 to 52 µg/m³ (median: 4 µg/m³). Occupational hygienists evaluated the exposure to TDI and MDI for each calendar year up to 1987 according to categories for each workplace and work task (no, low or intermittent exposure). Exposure after 1987 was not included. Over the total period, 1679 members of the cohort were exposed. No increase in mortality caused by malignant tumours overall or by obstructive lung diseases was observed. In women, mortality from lung tumours was increased with statistical significance (SMR: 3.52; 95% CI: 1.69–6.48). Six of the 12 new cases of lung cancer occurred in the group with no or low exposure (standardized incidence ratio (SIR): 4.00) and 3 in the group with exposure for more than 5 years (SIR: 4.76). An additional nested case–control study did not find an association between PU dust and lung cancer in women (Mikoczy et al. 2004).

A cohort study in the United States evaluated the mortality of 4545 workers who were employed at 4 PU-producing plants at some point from the beginning of PU production (between 1958 and 1965) and the closure of one of the plants (1982) or at the point in time when the data were originally collected (1984). The workers were employed for at least 3 months in departments or jobs that involved exposure. The TDI concentrations decreased markedly over time at all plants; at one plant, for example, levels declined from 360 µg/m³ in 1965 to 15 to 20 µg/m³ in 1982. The data for the vital status were updated until the end of 2011. NIOSH industrial hygienists developed a time-specific exposure matrix for TDI based on historical records and the exposure determinations that had been carried out in 3 of the 4 plants from 1984 to 1985 for the first follow-up study. The entire workforce was included in the exposed group at plant A; the matrices for plants B and D were operation-specific and the matrix for plant C was department-specific; each of these matrices combined 4 to 6 categories. On the basis of these exposure matrices and the work histories of the cohort members, cumulative exposure to TDI was estimated in µg/m³ × day and lagged 10 years for the analysis. The work history details necessary for this estimate were not available for 376 cohort members. Another 530 cohort members were still exposed at the time when the work history data were collected; however, only the exposure data for the years up to 1984 were included in the analysis. Several sensitivity analyses were carried out (use of state records instead of national records for reference; exclusion of cohort members with a duration of employment of less than 1 year; assumption of continuing exposure until the age of 65 for those cohort members who were still exposed at the workplace in 1984). In addition, the analysis included the length of time spent working in finishing jobs. More than 50% of the cohort members were employed and exposed for less than 1 year. The lung cancer mortality was increased with statistical significance (men: SMR: 1.30; 95% CI: 1.01–1.64; women: SMR: 2.27; 95% CI: 1.70–2.96); the SMR was 1.78 after short-term workers were excluded. The SMRs for lung cancer after the lagged exposure period and cumulative exposure increased across the first 3 quartiles and then decreased in quartile 4 although the increase was still statistically significant. Sex-specific analyses showed a similar pattern for men and women, but the SMRs for women were much higher. After short-term cohort members had been excluded, there was no evidence of an association between new cases of lung cancer and the lagged exposure period or cumulative exposure. A statistically significant association was found between new cases of lung cancer and the lagged employment period in finishing jobs, but not for the lagged employment duration in finishing jobs involving cutting tasks. Almost all women who died from lung cancer worked in finishing jobs; this was true for only a few men. In addition, statistically significant increases in mortality were reported for cancer of the larynx, breast cancer, brain tumours and non-Hodgkin's lymphomas (Pinkerton et al. 2016).

Summary: In all three cohort studies, the lung cancer risk was consistently found to be increased with statistical significance in workers of PU-producing plants. This was particularly evident among the women. Although there are no data available for individual smoking habits, it is unlikely that the increased risks can be explained by smoking habits. The lung cancer risks were not found to be associated with the duration of employment or cumulative exposure to TDI. This finding may have resulted from shortcomings in the method used to estimate exposure, the lack of adjustment for smoking habits and a healthy worker survivor effect. The association with the duration of employment

in finishing jobs may be evidence of the importance of dermal exposure, which was not taken into account in any exposure estimate.

5 Animal Experiments and in vitro Studies

5.1 Acute toxicity

Data for acute toxicity were described in detail in the 1999 supplement (Greim 1999). A new study is described below.

In an extensive study that investigated the sensitizing effects on the airways, groups of 4 Brown Norway rats or Wistar rats (not sensitized) were exposed nose-only to a 2,4-TDI/2,6-TDI mixture (80% : 20%) at concentrations of 0, 5, 21, 81, 110 or 113 mg/m^3 (690, 2898, 11178, 15180 and 15594 µl/m^3) for 30 minutes and then observed for 15 minutes. The respiratory minute volume and breathing frequency were monitored. Within the 30-minute exposure period, both parameters decreased by 20% to 75% in a concentration-related manner. Up to a concentration of 21 mg/m^3, the levels determined in Brown Norway rats for both parameters had returned to the range of those of the control group. After the 15-minute observation period, the values found in Wistar rats for both parameters were still decreased in comparison with those of the control animals. In the concentration group exposed to 21 mg/m^3, the percentage values for the respiratory minute volume were in the range of those of the 5 mg/m^3 group, but did not reach 100% of the initial level. These effects were considered to be evidence that the alveolar receptors are stimulated by irritation (Pauluhn 2014).

5.2 Subacute, subchronic and chronic toxicity

Groups of 4 Swiss Webster mice were exposed to 2,4-TDI concentrations of 7 to 1180 µl/m^3 for 3 hours a day on 5 days a week. The breathing frequency decreased at concentrations of 2,4-TDI of 23 µl/m^3 and above. Within the 5-day period of exposure, this response was found to increase with the exposure duration. The mice did not recover overnight. The respiratory rate decreased to 20% in a concentration-related manner. Histopathological changes were not observed following exposure to 31 µl/m^3 for 3 hours a day for 3 days (Sangha and Alarie 1979).

Groups of 8 male C57BI/6 mice were exposed to a 2,4-TDI/2,6-TDI mixture (80% : 20%) in concentrations of 0, 1.1, 1.5 or 2.4 mg/m^3 (160, 210 and 340 µl/m^3) for 1 hour a day on 5 days a week. The breathing frequency, break after inspiration (TB), pause after expiration (TP), time of inspiration (TI), time of expiration (TE), mid-expiratory flow (VD) and the tidal volume (VT) were determined as parameters of sensory and pulmonary irritation. TB, TI, TE, VT and VD increased and the breathing frequency decreased at the low concentration and above. On day 1, the effects were most pronounced in the 2 low concentration groups. In the high concentration group, the severity of the effects increased with time (Lindberg et al. 2011).

Sprague Dawley rats and CD-1 mice were exposed to a 2,4-TDI/2,6-TDI mixture (80% : 20%) in concentrations of 0, 50 or 150 µl/m^3 for 6 hours a day, on 5 days a week, for 2 years. In mice, necrotic and chronic rhinitis, sinusitis and irritant effects on the lower respiratory airways such as pneumonitis and bronchitis were observed in the 2 concentration groups. After exposure to 150 µl/m^3, laryngitis, tracheitis and irritant effects on the eyes (keratitis) were additionally observed in the male animals. Irritation was more severe compared with that found in the female animals. The incidences for rhinitis of varying levels of severity are shown in Table 5 (Hazleton Laboratories 1986). As rhinitis was observed in all mice at varying levels of severity at concentrations of 50 µl/m^3 and above, a NOAEC cannot be derived for this end point and a benchmark calculation cannot be carried out. In rats, rhinitis of varying grades of severity was likewise observed at the low concentration and above. The rhinitis was mainly observed in the frontal region of the nasal cavity. Unlike mice, rats were not found to have necrotic rhinitis or severe damage to the nasal epithelium (severity: 4). In male rats, the incidences observed at 50 µl/m^3 were in the same order of magnitude as those in the control animals. Therefore, the authors regarded the concentration of 50 µl/m^3 to be the NOAEC for male rats. A NOAEC for female rats could not be derived. The incidences show that the female rats reacted with greater sensitivity than the male rats (Hazleton Laboratories 1984).

As it was not possible to obtain a NOAEC for female rats, a benchmark calculation was carried out. The report does not include the incidences for rhinitis in all planes of section without the animals of the interim examinations. In addition to the incidences given in Table 5 for rhinitis in female rats (all planes of section with interim examinations after 6, 12 and 18 months), the incidences for rhinitis in the frontal section of the nasal cavity were reported for the animals that survived until the end of the study (terminal) (control animals: 10/35; 50 µl/m³: 11/26; 150 µl/m³: 26/36). Using the incidences of rhinitis for all animals, a supralinear concentration–effect relationship was obtained (see Figure 1) which is problematic for the benchmark calculation. Therefore, the benchmark dose for an additional incidence of 5% (BMD_{05}) compared with that in the control group (extra risk) and the $BMDL_{05}$ (lower 95% confidence limit of the BMD_{05}) were calculated from the terminal data. The BMD_{05} is between 8 and 23 µl/m³ and the $BMDL_{05}$ is between 3 and 14 µl/m³. The logistic model was determined to be the best model (lowest AIC value); the p value is 0.97. A BMD_{05} of 13 µl/m³ and a $BMDL_{05}$ of 10 µl/m³ were derived by applying this model. These values are almost identical to those obtained using the probit model. The benchmark calculation for the end point "rhinitis in female rats" is shown in Figure 2.

Tab. 5 Incidences of the different grades of severity of rhinitis in rats (with interim examinations) and mice (without interim examinations) after 2-year inhalation exposure to a 2,4-TDI/2,6-TDI mixture (Hazleton Laboratories 1984, 1986)

	0 µl/m³				50 µl/m³				150 µl/m³			
	Rat		Mouse		Rat		Mouse		Rat		Mouse	
	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀	♂	♀
rhinitis												
grade 0	71	93	0	0	61	55	0	0	25	39	0	0
1	49	28	1	0	52	62	9	3	61	71	1	0
2	1	0	2	4	2	5	17	18	28	10	2	0
3	0	2	4	2	1	1	23	24	0	1	18	11
4	0	0	1	0	0	0	26	27	0	0	32	29
5	no data	no data	0	0	no data	no data	6	10	no data	no data	14	20
necrotic rhinitis	no data	no data	0	0	no data	no data	4	8	no data	no data	22	29
grades 1 to 5 and necrotic rhinitis	50/121	30/123	8/90	6/90	55/126	68/123	85/90	90/90	89/114	82/121	89/90	89/90
grades 1 to 5 in %	41	24	9	7	47	55	94	100	77	68	99	99

grade 0: no unusual findings, no inflammatory effects in the different sections;
grade 1: mild rhinitis, inflammation generally only in 1 section, epithelial changes, slight accumulation of leukocytes;
grade 2: moderate rhinitis, inflammation of the mucosa in 1 section or large areas in 2 sections, in addition, catarrhal inflammation;
grade 3: marked rhinitis, massive inflammation in all 3 sections of the nasal cavity;
grade 4: (mouse only) necrotic rhinitis, severe damage to the nasal cavity with necrosis and scars;
grade 5: (mouse only): severe necrotic rhinitis, deformation of the nasal turbinates, cause of death in several mice

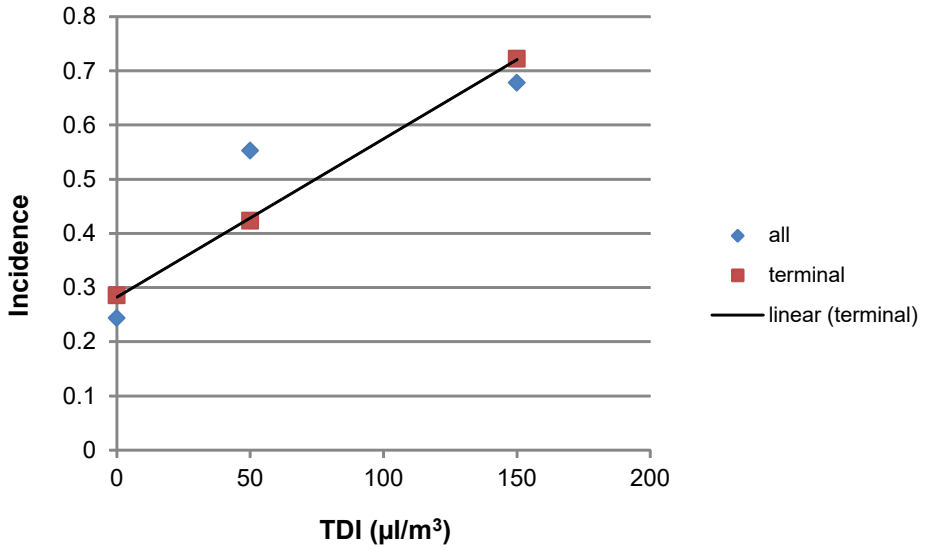

Fig. 1 Incidences for all grades of severity of rhinitis (1–3) in all parts of the nose of the female rats (more sensitive than males)

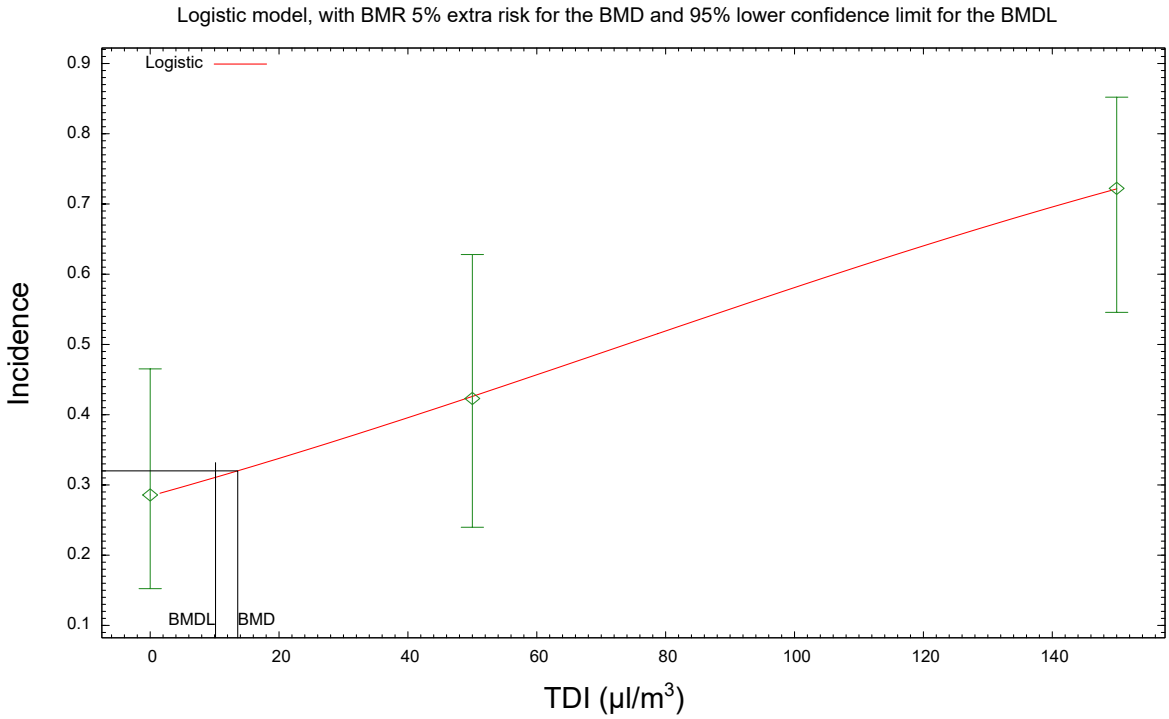

Fig. 2 Benchmark calculation for the end point “rhinitis in female rats”

5.3 Allergenic effects

5.3.1 Sensitizing effects on the skin

The sensitizing effects of TDI on the skin were described in detail in the supplement published in 2015 (Hartwig 2015).

5.3.2 Sensitizing effects on the airways

To date, no animal model has been validated for determining sensitizing effects on the airways. However, there are a large number of studies that evaluated intradermal and dermal routes of administration, inhalation exposure only or a combination of intradermal and inhalation induction. These findings can be used as supporting evidence for the data obtained in humans. Whereas the inhalation challenge with TDI only rarely led to specific immediate type reactions, corresponding reactions were reproducible after the challenge with, for example, TDI conjugated to guinea pig albumin. In animal studies, the topical application of TDI after intranasal or intratracheal re-exposure led to airway reactions, inflammatory changes of the bronchial mucosa or increases in the total or TDI-specific IgE (for example Ban et al. 2006; Fukuyama et al. 2008; Selgrade et al. 2006; Tarkowski et al. 2007; Vanoirbeek et al. 2004, 2008, 2009 a, b).

5.3.2.1 Inhalation

It was possible to derive concentration–effect relationships for repeated induction by inhalation from a number of studies; the minimum effective concentrations of TDI were between 20 and about 400 µl/m³ (0.15–2.6 mg/m³) with NOECs between 2 and 30 µl/m³ (0.015–0.23 mg/m³). In animals, the specific respiratory sensitization is often accompanied by non-specific bronchial hyperreactivity (Schupp and Collins 2012; see also Greim 1999).

In a modified local lymph node assay with inhalation exposure carried out to investigate substances that cause sensitizing effects on the airways, groups of 6 BALB/c mice were exposed to TDI at an average concentration of 7.4 mg/m³ (purity: 80%) on 3 consecutive days for 45, 90, 180 or 360 minutes a day. Three days after the last exposure, mandibular and auricular lymph nodes were removed and the lymphocyte proliferation was determined following ex vivo labelling with [³H]-thymidine. The stimulation index for the lymphocytes of the auricular lymph nodes was found to be more than three times the value in the control group only in the group exposed for 180 minutes. A similar increase in the stimulation index for the lymphocytes of the mandibular lymph nodes was already detected in the group with the shortest duration of exposure (Arts et al. 2008).

For induction, female C57BL/6 mice were exposed by inhalation to a 2,4-TDI/2,6-TDI mixture at a concentration of 50 µl/m³ for 4 hours a day on 12 consecutive days. One day later, total IgE, TDI-specific IgG and various cytokines were determined in the animals (group A). In the second group that was pre-treated in the same way, these parameters were determined 2 weeks after the induction treatment (group B). The third group (C) was treated for the challenge with the same concentration for 4 hours a day on 3 consecutive days following a 2-week recovery period. The 2 control groups consisted of mice exposed to air only (group D) and mice exposed to a 2,4-TDI/2,6-TDI mixture at the challenge only (group E). Increased incidences of eosinophils in the nasal cavity were found in the animals of groups A and C compared with the incidences determined in group B and the control groups. Cytokines IL-4, IL-5, IL-13 and IFN-γ were increased in the animals of these groups compared with the levels found in group D or group E. Immediately after the last induction treatment, the baseline enhanced pause (Penh) and the inspiratory time were increased in the animals of group A and the respiratory rates were decreased compared with the values of the animals of group D (Johnson et al. 2007).

For the induction treatment by inhalation, female C57BL/6 mice were exposed nose-only to a 2,4-TDI/2,6-TDI mixture (80% : 20%) at a concentration of 20 µl/m³ for 6 weeks (4 hours a day on 5 days a week). Two weeks later, the mice were challenged with this mixture for 1 hour. After 1 day, the airway hyperreactivity (Penh after inhalation of methacholine at a concentration of 50 mg/ml) was markedly increased in the sensitized animals compared with the values of 3 control groups (exposure to air only, challenge treatment only and sensitized animals without challenge treatment). Total IgE was about 10 times as high as the levels in the control animals; no statistically significant effect on this parameter was observed after a single 2-hour exposure to a concentration of 500 µl/m³. However, TDI-specific IgG was increased both in the animals with subacute exposure and in those with one pre-treatment. The infiltration of lymphocytes, neutrophils and eosinophils was observed in the nose and lungs of the animals with 6-week induction treatment. The secretion of IL-4, IL-5, IFN-γ and TNF-α was increased only in the group with subacute exposure (Matheson et al. 2005 b).

Groups of 11 to 12 male Hartley guinea pigs were exposed nose-only for induction to 2,4-TDI or 2,6-TDI in concentrations of 1290 to 1400 µl/m³ for 3 hours on 5 consecutive days. Two weeks after induction, the animals began a 3-week treatment regimen involving exposure once a week for 1 hour to either the isomer used for induction or the other isomer (first and second TDI challenges). The isomers were administered in concentrations of 18 to 46 µl/m³. In week 3, the animals were challenged with the aerosol of a TDI guinea pig albumin conjugate. Irritation of the respiratory tract was observed only during the induction phase. During the first challenge, the incidences of immediate reactions (determined on the basis of the increase in the respiratory rate) were not higher in the pre-treated animals than in the control animals (a maximum of 1 or 2 animals per group). After the second challenge with TDI and the conjugate, reactions were observed in the individual groups in 1 to 5 and 4 to 5 of the pre-treated animals and in a maximum of 1 control animal; differences between the 2 isomers were not found. Delayed reactions after 2 to 16 hours were observed also in up to 80% of the control animals (Shiotsuka et al. 2000); therefore, the findings are not conclusive.

5.3.2.2 Consecutive exposure (dermal – via airways)

An extensive study was carried out with 6 groups of 4 Brown Norway rats. On day 1 of the main study, all 6 groups were treated dermally for induction with 100 µl of a 2% formulation of a 2,4-TDI/2,6-TDI mixture (80% : 20%) in acetone. Seven days later, the animals were treated again using the same formulation. Two weeks after the dermal induction treatment, the animals of 3 groups (A1–A3) were first primed by inhalation exposure to a 2,4-TDI/2,6-TDI mixture in concentrations of 80 to 85 mg/m³ (11 040–11 730 µl/m³) 3 times for 30 minutes at intervals of 2 weeks. Another 2 weeks later, the animals were challenged with the same concentration, but for 10 (A1), 30 (A2) or 60 (A3) minutes. In the remaining 3 groups (B1–B3), the latter challenge treatment was carried out 2 weeks after dermal induction without first priming the animals. Lung function parameters (whole body plethysmography), exhaled nitrogen monoxide (eNO) and the analysis of neutrophilic granulocytes in the BALF were evaluated as end points. In the BALF obtained 1 day after the last challenge treatment, neutrophilic granulocytes were increased in the groups with statistical significance ($p < 0.01$) and in a concentration-related manner compared with the values of the control group that had been dermally pre-treated with the vehicle; this increase was most pronounced in group A3. In groups A2, B2 and B3, neutrophilic granulocytes were increased without statistical significance compared with the values in the control animals. In addition, in group A3, eNO (about 20 hours after the challenge treatment), lung weights and the protein levels in the BALF were increased with statistical significance. Furthermore, the protein level in the BALF was increased in group A1 and eNO was increased in groups A1 and A2 compared with the values in the control animals ($p < 0.05$). In group A3 (but not in group A1 or A2), Penh (as a measure of the duration of apnoea) was increased; the maximum value was determined about 3 to 5 hours after the challenge treatment. A NOAEC of about 0.02 mg/m³ (3 µl/m³) was derived on the basis of the value of 1000 mg/m³ × minute obtained for c × t after exposure to a 2,4-TDI/2,6-TDI mixture for 10 minutes, taking into account correction factors for the interindividual susceptibility of humans (factor of 10), the rat-to-human adjustment (a factor of 3 for considering obligatory nasal breathing in rats compared with humans and a factor of 3 for varying sensitivity of the species) and assuming 8-hour exposure at the workplace (1000 mg/m³ × min × 1/480 min × 1/(10 × 3 × 3)) (Pauluhn 2014).

On days 1 and 7, Brown Norway rats were treated topically with 1% TDI (no other details) in acetone/olive oil. After 2, 4 and 6 weeks, the animals were exposed by inhalation to TDI at a concentration of 70 mg/m³ (9660 µl/m³) for 30 minutes. Another 2 weeks later, the animals were once again challenged with TDI for 30 minutes using concentrations of 1.5, 6, 20 or 70 mg/m³. A TDI concentration of 6 mg/m³ was determined to be the NOAEC for the initiation of a reaction based on the histopathological examinations carried out on the following day, the analysis of the BALF and the weights of the lung-associated lymph nodes (Schupp and Collins 2012).

A study in mice with dermal induction and nasal provocation found that the allergic reaction was mediated by the B lymphocytes; however, IgE and T lymphocytes or the cytokines that they secreted were not involved (De Vooght et al. 2013; Haenen et al. 2015).

Male BALB/c mice were sensitized by topical pre-treatment with 1% 2,4-TDI (in acetone/olive oil, 4:1; 100 µl on the abdomen and paws) twice daily on 2 consecutive days; the intratracheal challenge 1 week later with glutathione TDI

adducts (50 µl solution with a TDI level equivalent to 150 µg) led to increases in neutrophils in the lung lavage fluid. Similar effects were observed in naive animals that were treated with about twice the amount of conjugated TDI (Valstar et al. 2004).

On days 1 and 8, male BALB/c mice were treated dermally with 0.5% 2,4-TDI at a concentration of 20 µl/ear or with the vehicle control acetone:olive oil (3:2). On day 15 and thereafter, the mice received intranasal instillations of 40 µl of 0.1% 2,4-TDI once a day, on 5 days a week, for 5 weeks. One day after the last instillation, the hyperreactivity to methacholine and the lungs were examined and parameters of inflammation were determined. Immunological parameters were analysed in the BALF, lung tissue, blood and lymph nodes. The following 3 groups were evaluated: control group (without 2,4-TDI treatment), vehicle/2,4-TDI and 2,4-TDI/2,4-TDI (dermal/intranasal). In both treatment groups, significant increases in dendritic cells (CD11b+), T cells and B cells were recorded in the lymph nodes. The total leukocyte count was not increased. The CD3+ helper cells were not increased in the lungs of the animals of the 2 treatment groups compared with control values. The ex vivo stimulation of (auricular) lymphocytes of the 2 treatment groups yielded statistically significant increases in IL-4, IL-10, IL-13 and IFN-γ. In the serum, IL-13 and IgE were increased with statistical significance compared with control levels; the increase was highest in the 2,4-TDI/2,4-TDI group. The airway hyperreactivity was markedly more pronounced in the 2,4-TDI/2,4-TDI group than in the vehicle/2,4-TDI group or in the control group. The number of neutrophils, eosinophils or lymphocytes was not increased in the BALF of the 2,4-TDI/2,4-TDI group. However, the histopathological examination yielded a limited increase in inflammation around the blood vessels, which was characterized by macrophages, monocytes and leukocytes. The authors explained that the Th2 hypersensitivity and airway hyperreactivity induced by the combination of dermal application and subsequent exposure via the airways were markedly more pronounced than after exposure via the airways alone. This was mainly a Th1/Th2 systemic response without hyperreactivity of the airways (Pollaris et al. 2019).

5.3.2.3 Other experimental findings

Various animal models in mice yielded evidence of the secretion of IL-4, a number of other interleukins and INF-γ (for example Fukuyama et al. 2008; Johnson et al. 2007; Matheson et al. 2005 a; Tarkowski et al. 2007, 2008; Vandebriel et al. 2000; Vanoirbeek et al. 2008), whereas only or mainly Th2 cytokines were expressed in other studies (Plitnick et al. 2005; Selgrade et al. 2006).

In BALB/c mice sensitized by nasal administration of a 3% TDI aerosol (no other details), increases in eosinophils and neutrophils and a time-dependent increase in the vascular endothelial growth factor (VEGF) were observed in the BALF 6 to 72 hours after the challenge with a 1% TDI formulation (Lee et al. 2002).

Regulatory T lymphocytes of BALB/c mice treated topically once with 2,4-TDI reduced the proliferation of T lymphocytes ex vivo to a greater extent than the corresponding cells of naive mice (Long et al. 2016).

In bronchial epithelial cells of humans (BEAS-2B), TDI (no other details) applied in concentrations of 0.25 to 1 mM in vitro led to the expression of menthol receptor 1 (CMR1, transient receptor potential melastatin 8, TRPM8) and the interleukins IL-4, IL-13, IL-25 and IL-33. Their secretion was again inhibited to some extent by the TRPM8 antagonist BCTC (N-(4-tert-butylphenyl)-4-(3-chloropyridin-2-yl) piperazine-1-carboxamide) (Kim et al. 2017).

5.4 Reproductive and developmental toxicity

The 2-generation study and the developmental toxicity study were reviewed as unpublished studies in the 1999 supplement (Greim 1999). They are now published. No recent data are available.

5.4.1 Fertility

In a 2-generation study carried out according to OECD Test Guideline 416 (from 1983), groups of 28 Sprague Dawley rats per sex were exposed whole-body to TDI vapour in concentrations of 0, 20, 80 or 300 µl/m^3 for 6 hours a day, 5 days per week for 10 weeks, then mated within groups for 3 weeks, with exposure on 7 days per week during mating, gestation and lactation. The test substance consisted of a 80 : 20 mixture of the 2,4 and 2,6 isomers with a purity of more

than 99%. Exposure of the male animals continued only until the birth of the litter, while the female animals were exposed until day 19 of gestation and additionally from days 5 to 20 after birth. Starting on day 28 after birth, animals of the F1 generation were exposed and bred as described above. The treatment had no effects on mating success, the reproductive organs, gestation or the index of live births, the survival of the offspring or the body weights per litter of the offspring of the F0 and F1 generations. Minimal to moderate rhinitis was observed in the adult animals of the F0 and F1 generations at the lowest concentration tested of 20 µl/m^3. Both the incidences and the severity increased with the concentration. In addition, dysplasia or hyperplasia was observed in the nasal cavity of the animals of the high concentration group. At concentrations of 80 µl/m^3 and above, in a few cases body weights and body weight gains were reduced and red discoloration of the fur was observed. In the F2 generation, body weight gains were reduced during lactation in the 2 high concentration groups, whereas the birth weights and body weights were not decreased up to postnatal day 4. The NOAEC for fertility was 300 µl/m^3 for the 2,4-TDI/2,6-TDI mixture. A NOAEC for parental toxicity was not derived because adverse effects were observed even in the low concentration group (see Greim 1999; Tyl et al. 1999 b). The NOAEC for perinatal toxicity was 300 µl/m^3 for the 2,4-TDI/2,6-TDI mixture because no effects were observed in the offspring up to postnatal day 4.

5.4.2 Developmental toxicity

In a prenatal developmental toxicity study carried out according to US EPA guidelines valid in 1985, which are similar to OECD Test Guideline 414, groups of 25 Sprague Dawley rats were exposed whole-body to a 2,4-TDI/2,6-TDI mixture for 6 hours a day from days 6 to 15 of gestation in concentrations of 0, 20, 100 or 500 µl/m^3. The test substance was the same as that used in the generation study. The animals were examined on day 21 of gestation. After exposure to the 2,4-TDI/2,6-TDI mixture at a concentration of 500 µl/m^3, reduced body weights and body weight gains, reduced feed consumption, nasal discharge and laboured breathing were observed in the dams. Exposure to the 2,4-TDI/2,6-TDI mixture had no effects on pre-implantation or post-implantation losses, the sex ratio or foetal weights per litter. Teratogenic effects were not observed. After exposure to the 2,4-TDI/2,6-TDI mixture at a concentration of 500 µl/m^3, only the incidence of incomplete ossification of the 5th cervical vertebra was increased in the foetuses (foetuses: 36/154; 23.4%; control group: 13/156; 8.3%; litter: 18/23; 78.3%; control group: 8/22; 36.4%). The incidence of all skeletal variations was not changed with statistical significance. The NOAEC for developmental and maternal toxicity was determined to be 100 µl/m^3 for the 2,4-TDI/2,6-TDI mixture (Greim 1999; Tyl et al. 1999 a).

External malformations were not observed at the necropsy of the offspring of the F0 and F1 generations from the 2-generation study described above (Greim 1999; see also Section 5.4.1; Tyl et al. 1999 b). The NOAEC for perinatal toxicity was determined to be 300 µl/m^3 for the 2,4-TDI/2,6-TDI mixture.

5.5 Genotoxicity

5.5.1 In vitro

As described in the supplement published in 1999 (Greim 1999), the positive results obtained in bacteria and mammalian cells are attributed to the secondary or decomposition and reaction products of TDI. Particularly in tests using solvents containing water such as DMSO, hydrolysis can occur within a short period of time, even at concentrations usually applied in the Salmonella mutagenicity test. Hydrolysis leads to the formation of the aromatic amine TDA and numerous other secondary products.

5.5.2 In vivo

A large number of studies are available that investigated the clastogenic effects induced by TDI in test animals after inhalation exposure. These studies did not reveal genotoxic effects in the lungs or in the bone marrow (see Greim 1999). Likewise, micronuclei in the bone marrow or in the peripheral blood were not induced in a micronucleus test with male C57Bl/6J mice that were exposed to a 2,4-TDI/2,6-TDI mixture (80% : 20%) in concentrations up to 340 µl/m^3 for 1 hour on 5 consecutive days. The ratio of polychromatic to normochromatic erythrocytes was slightly reduced

during the first 3 days depending on the cumulative inhaled concentration (Lindberg et al. 2011). A study that reported a statistically significant increase in chromosomal aberrations and SCE in the bone marrow of exposed mice (Ji et al. 2008) cannot be evaluated because the concentrations used were not given (only: 1/4 or 1/2 of the LC_{50}). Exposure was carried out for 4 hours a day on 14 consecutive days.

A sex-linked recessive lethal (SLRL) test with male Drosophila melanogaster and a test for reciprocal translocations yielded positive results (ATSDR 2018).

Altogether, the available in vivo studies did not demonstrate clastogenic effects in the lungs or in the bone marrow of the exposed animals. Neither DNA adducts nor UDS were induced in the liver of rats. Studies that investigated the mutagenicity in mammals are not available. Mutations were induced in Drosophila (ATSDR 2018).

5.6 Carcinogenicity

5.6.1 Short-term studies

2,4-TDI was investigated in various short-term tests, which were described as indicator tests for carcinogenic effects of organic chemicals (cell transformation, degranulation of the endoplasmic reticulum (ER) in the liver, sebaceous gland suppression test, tetrazolium reduction test and subcutaneous implantation test in mice). Only the last test system yielded positive results. After 1 mmol of 2,4-TDI was suspended in 10 ml of melted pig gelatine, 0.2 ml aliquots were placed on a Millipore filter. These filters were then implanted subcutaneously in 10 mice of both sexes. After 3 months, the tissue surrounding the filters was examined histopathologically; the differences from the tissues of the control group were interpreted as tumours (no other details) (Purchase et al. 1978; Styles 1978). The usefulness of these short-term tests is limited because the tests were not validated.

A rat liver foci test with TDI (no other details) did not reveal increases in pre-neoplastic cell foci and thus no evidence of tumour-initiating effects after inhalation exposure of rats to a concentration of 2 µl/m³ on 5 days a week for 4 weeks followed by treatment with the tumour promoter Clophen A50 for 8 weeks (Wiethege et al. 1995).

5.6.2 Long-term studies

No new long-term studies with 2,4-TDI, 2,6-TDI or the mixture have become available since the publication of the supplement in 1999 (Greim 1999). The findings of the 2-year inhalation study and the 2-year study after oral administration are discussed below and compared with the carcinogenic effects of the metabolite 2,4-TDA.

5.6.2.1 Inhalation exposure

SD rats and CD-1 mice were exposed to a 2,4-TDI/2,6-TDI mixture in concentrations of 0, 50 or 150 µl/m³ for 6 hours a day, on 5 days a week, for 2 years. In the animals treated with TDI, variations or slight increases in the tumour incidences were observed for some locations compared with the incidences in the control animals. Table 6 lists all tumour types for which at least 2 more tumour-bearing animals in the TDI group were observed than in the concurrent control group. The tumour spectrum was in the normal range in all groups; therefore, TDI was not regarded as carcinogenic (Hazleton Laboratories 1980, 1984, 1986; Loeser 1983). However, the authors did not carry out a statistical analysis of the results. Several tumour incidences differed from those in the concurrent control animals. The incidence of lung adenomas was increased in the male mice exposed to TDI, although not in a concentration-related manner, and lymphomas were increased in the middle concentration group. However, the CD1 mouse is known to have a high spontaneous tumour rate and high variability in the findings, particularly at these two locations (Maita et al. 1988; Sher et al. 1982).

On the basis of these findings, exposure by inhalation did not lead to carcinogenic effects in rats or mice.

Tab. 6 Carcinogenicity studies after inhalation exposure to a 2,4-TDI/2,6-TDI mixture

Author:	Hazleton Laboratories 1980, 1984; Loeser 1983			
Substance:	2,4-TDI/2,6-TDI mixture (80%:20%)			
Species:	**rat** (Sprague Dawley CD), groups of 104–105 per sex; in addition, 7 animals/sex/group/interim sacrifice after 6, 12 and 18 months			
Administration route:	whole-body exposure to TDI vapour, 6 hours/day, 5 days/week			
Concentration:	0, 50, 150 µl/m³			
Duration:	♂: 110 weeks; ♀: 108 weeks			
Toxicity:	no clinical signs, decreases in body weights only until test week 12 in the groups exposed to 150 µl/m³, no effects on the blood count, clinical chemistry, urinalysis or organ weights, slight concentration-dependent increase in rhinitis in the frontal section of the nasal cavity (see Table 4)			
		Concentration [µl/m³]		
		0	**50**	**150**
surviving animals (after 104 weeks)	♂	37/104 (36%)	34/104 (33%)	30/104 (29%)
	♀	35/104 (34%)	25/105 (24%)	38/105 (36%)
types of tumours affecting at least 2 more animals in the groups treated with TDI than in the control group:				
adenoma (skin)	♂	0/104	0/104	3/104 (3%)
papilloma (skin)	♀	1/104 (1%)	3/105 (3%)	3/105 (3%)
benign tumour (mammary gland)	♀	24/104 (23%)	27/105 (26%)	22/105 (21%)
carcinoma (mammary gland)	♀	9/104 (9%)	9/105 (9%)	14/105 (13%)
fibroma (subcutis/muscle/bone)	♂	29/104 (28%)	22/104 (21%)	35/104 (34%)
	♀	1/104 (1%)	1/105 (1%)	4/105 (4%)
histiocytoma (subcutis/muscle/bone)	♂	1/104 (1%)	4/104 (4%)	1/104 (1%)
malignant lymphoma (haematopoietic system)	♀	1/104 (1%)	1/105 (1%)	3/104 (3%)
haemangioma (haematopoietic system)	♂	1/104 (1%)	1/104 (1%)	4/104 (4%)
islet cell adenoma (pancreas)	♂	1/104 (1%)	2/104 (2%)	3/104 (3%)
adenoma (pituitary gland)	♀	64/104 (62%)	62/105 (59%)	67/105 (64%)
meningioma (brain)	♂	0/104	0/104	2/104 (2%)
lipomatous tumour (kidneys)	♂	1/104 (1%)	3/104 (3%)	0/104
Author:	Hazleton Laboratories 1986; Loeser 1983			
Substance:	2,4-TDI/2,6-TDI mixture (80%:20%)			
Species:	**mouse** (CD-1), groups of 90 per sex; in addition, 10 animals/sex/group/interim sacrifice after 6, 12 and 18 months			
Administration route:	whole-body exposure to TDI vapour, 6 hours/day, 5 days/week			
Concentration:	0, 50, 150 µl/m³			
Duration:	104 weeks			
Toxicity:	mortality slightly increased in ♀, decreases in body weight gains in the group given 150 µl/m³, no effects on the blood count, clinical chemistry or urinalysis; in both concentration groups, chronic necrotic rhinitis, sinusitis and irritant effects on the lower respiratory airways (pneumonitis, bronchitis), in ♂ of the group given 150 µl/m³ additionally laryngitis, tracheitis, irritant effects on the eyes (keratitis); irritant effects more pronounced in ♂ (see Table 5)			

Tab. 6 *(continued)*

		Concentration [µl/m³]		
		0	**50**	**150**
surviving animals (after 104 weeks)	♂	25/90 (28%)	34/90 (38%)	34/90 (38%)
	♀	40/90 (44%)	27/90 (30%)	28/90 (31%)
types of tumours affecting at least 2 more animals in the groups treated with TDI than in the control group:				
adenoma/microadenoma (lungs)	♂	17/90 (19%)	22/90 (24%)	16/90 (18%)
adenoma (multiple) (lungs)	♂	1/90 (1%)	9/90 (10%)	6/90 (7%)
adenoma (total) (lungs)	♂	18/90 (20%)	31/90 (34%)	22/90 (24%)
lymphoma (haematopoietic system)	♂	2/90 (2%)	12/90 (13%)	5/90 (6%)
leukaemia (haematopoietic system)	♀	1/90 (1%)	3/90 (3%)	0/90

historical control data: lung adenomas: ♂ 17% (0%–38%), ♀ 14% (0%–41%) (Sher et al. 1982)
lung adenocarcinomas: ♂ 5% (0%–16%), ♀ 3% (0%–12%) (Sher et al. 1982)
lymphoreticular tumours: ♂ 6% (0%–16%), ♀ 11% (3%–22%) (Sher et al. 1982), ♂ 8.8%, ♀ 22% (Maita et al. 1988)

5.6.2.2 Oral exposure

Gavage doses of a 2,4-TDI/2,6-TDI mixture (80%:20%) in corn oil were given to F344 rats and B6C3F1 mice (Table 7). Increased incidences of tumours were observed in the subcutaneous tissue and in the pancreas of rats of both sexes. Nodular changes in the liver and mammary gland tumours were observed only in the treated female animals. In the female mice of the high dose group, the incidences of haemangiomas, haemangiosarcomas and liver adenomas were increased with statistical significance. The treatment-related increases in mortality that were observed except in the female mice and the up to 30% decreases in body weight gains indicate that the maximum tolerated dose was exceeded in both species. The tumour spectrum corresponded to that observed in an earlier study with 2,4-TDA, the hydrolysis product of 2,4-TDI (see Table 8). TDI was not carcinogenic in male mice. Even before administration, 10% to 23% of the TDI had been broken down as a result of the residual moisture in the corn oil (about 0.05%) and because the formulated test substance was stored for up to 8 days before use. Therefore, the administered sample contained also polyurea and other unidentified decomposition or reaction products of TDI. The carcinogenic effects that are induced by TDI after oral administration are assumed to be caused by the 2,4-TDA and 2,6-TDA salts that form once TDI enters the acidic milieu of the stomach (Dieter et al. 1990; NTP 1986).

Tab. 7 Carcinogenicity studies after oral administration of a 2,4-TDI/2,6-TDI mixture

Author:	Dieter et al. 1990; NTP 1986
Substance:	2,4-TDI/2,6-TDI mixture (80%:20%)
Species:	**rat**, F344/N, groups of 50 ♂, 50 ♀
Administration route:	gavage, in corn oil, 5 days/week
Dose:	♂: 0, 30, 60 mg/kg body weight and day, ♀: 0, 60, 120 mg/kg body weight and day, target dose reduced by 10% to 23% because TDI reacted with moisture in corn oil
Duration:	106 weeks
Toxicity:	decreases in body weights in week 10 (♂) and week 20 (♀) and thereafter, mortality increased in both dose groups, increased incidence of acute bronchopneumonia in both dose groups

Tab. 7 *(continued)*

		Dose [mg/kg body weight and day]		
		0	**30/60**	**60/120**
surviving animals (after 106 weeks, control animals: 87 weeks)	♂	36/50 (72%)	14/50 (28%)	8/50 (16%)
	♀	36/50 (72%)	19/50 (38%)	5/50 (10%)
tumours and pre-neoplastic changes				
fibromas (subcutis)	♂	3/50 (6%)#	3/50 (6%)	**9/50 (18%)***
	♀	0/50#	1/50 (2%)	3/50 (6%)
fibrosarcomas	♂	0/50	3/50 (6%)	3/50 (6%)
	♀	2/50 (4%)	0/50	2/50 (4%)
mammary gland: adenomas	♀	17/50 (34%)	25/50 (50%)	21/50 (42%)
pancreas				
nodular hyperplasia	♂	0/47	2/47 (4%)	4/47 (9%)
acinar cell adenomas	♂	1/47 (2%)#	3/47 (6%)	**7/49 (14%)***
islet cells, adenomas and carcinomas	♂	1/47 (2%)#	0/47	**4/49 (8%)***
islet cells, adenomas	♀	0/50	**6/49 (12%)***	2/47 (4%)
liver, neoplastic nodules	♀	3/50 (6%)#	**8/50 (16%)***	**8/48 (16%)***
Author:		Dieter et al. 1990; NTP 1986		
Substance:		2,4-TDI/2,6-TDI mixture (80% : 20%)		
Species:		**mouse**, B6C3F1, groups of 50 ♂, 50 ♀		
Administration route:		gavage, in corn oil, 5 days/week		
Dose:		♂: 0, 120, 240 mg/kg body weight and day, ♀: 0, 60, 120 mg/kg body weight and day, target dose reduced by 10% to 23% because TDI reacted with moisture in corn oil		
Duration:		106 weeks		
Toxicity:		decreases in body weights in both dose groups (♂) and in the high dose group (♀), only ♂: mortality in both dose groups increased, cytomegaly of the renal tubular cells		
		Dose [mg/kg body weight and day]		
		0	**120/60**	**240/120**
surviving animals (after 107 weeks, control animals: 87 weeks)	♂	46/50 (92%)	40/50 (80%)	26/50 (52%)
	♀	34/50 (68%)	43/50 (86%)	33/50 (66%)
tumours and pre-neoplastic changes				
liver adenomas	♀	2/50 (4%)#	3/50 (6%)	**12/50 (24%)***
liver carcinomas	♀	2/50 (4%)	2/50 (4%)	3/50 (6%)
haemangiosarcomas	♀	0/50	0/50	3/50 (6%)
haemangiomas and haemangiosarcomas	♀	0/50#	1/50 (2%)	**5/50 (10%)***
malignant lymphomas or leukaemia	♀	13/50 (26%)	17/50 (34%)	16/50 (32%)

*$p \leq 0.05$; #positive trend test

Tab. 8 Tumour incidences after administration of 2,4-TDA with the diet (NCI 1979; Reuber 1979)

		Dose [mg/kg body weight and day]		
F344 rat		**0**	**5.9**	**13**
surviving animals (after 78 weeks)	♂	18/20 (90%)	42/50 (84%)	32/50 (64%)
	♀	20/20 (100%)	46/50 (92%)	46/50 (92%)
neoplastic nodules and carcinomas (liver)	♂	0/20	5/49 (10%)	**10/50 (20%)***
	♀	0/20	0/50	6/49 (12%)
mammary adenomas and carcinomas	♂	0/20	5/50 (10%)	5/50 (10%)
	♀	1/20 (5%)	**38/50 (76%)***	**41/50 (82%)***
fibromas of the subcutis	♂	1/20	**15/50 (30%)***	**19/50 (38%)***
	♀	0/20	4/50 (8%)	**10/50 (20%)***
lipomas of the subcutis	♂	0/20	3/50 (6%)	8/50 (16%)
mesotheliomas	♂	0/20	5/50 (10%)	8/50 (16%)
pancreas				
acinar cell adenomas	♂	2/19 (11%)	10/42 (24%)	10/44 (23%)
	♀	0/19	0/45	3/41 (7%)
islet cells, adenomas	♂	0/19	2/42 (5%)	2/44 (5%)
phaeochromocytomas	♂	1/20 (5%)	4/49 (8%)	8/50 (16%)
lung tumours	♂	0/20	5/50 (10%)	5/50 (10%)
	♀	1/20	4/50 (8%)	3/50 (6%)
B6C3F1 mice		**0**	**15**	**30**
surviving animals (after 101 weeks)	♂	18/20 (90%)	45/50 (90%)	43/50 (86%)
	♀	15/20 (75%)	40/50 (80%)	39/50 (78%)
liver, carcinomas	♂	5/20 (25%)	17/50 (34%)	13/49 (27%)
	♀	0/19	**13/47 (28%)***	**18/46 (39%)***
lungs, carcinomas	♂	0/20	**9/50 (18%)***	6/49 (12%)
lymphomas or leukaemia	♂	2/20 (10%)	15/50 (30%)	8/49 (16%)
	♀	2/19 (11%)	**29/47 (62%)***	11/46 (24%)
haemangiomas/haemangiosarcomas	♂	2/20 (10%)	10/50 (20%)	10/49 (20%)
	♀	0/19	5/47 (11%)	3/46 (7%)

*$p < 0.05$

Summary: The findings obtained in animal studies were decisive for the classification of 2,4-TDI and 2,6-TDI in Carcinogen Category 3 A in 1999. After long-term oral administration of a 2,4-TDI/2,6-TDI mixture, the number of tumours in various organs that were attributed to TDA were increased in rats of both sexes and in female mice. No substance-induced tumours were found after inhalation exposure for 2 years. TDI was found to be genotoxic in vitro. Hydrolysis of TDI to form the metabolite 2,4-TDA is regarded as the mechanism responsible for the effects induced by TDI; this metabolite has genotoxic properties and was found to be carcinogenic in animal studies. Therefore, the carcinogenic risk associated with TDI depends greatly on the amount of TDA formed in vivo (see Table 8).

6 Manifesto (MAK value/classification)

Sensitizing effects and irritation are the most sensitive end points in humans and animals.

MAK value. TDI has pronounced sensitizing effects on the airways; these have been demonstrated both in animals and in humans. It is not possible to derive a concentration that provides protection from sensitization.

Irritation: An odour threshold between 10 and 20 µl/m^3 was derived from volunteer studies for TDI (Henschler et al. 1962). No conclusions about irritation were drawn from a volunteer study after exposure to TDI at a concentration of 5 µl/m^3 for 6 hours (Vandenplas et al. 1999). Eye irritation was reported by 2 of 49 workers who were exposed to

maximum concentrations of 5.4 to 10 µl/m³ (Gui et al. 2014). To date, concentrations of around 1 µl/m³ have not been found to cause irritation.

Lung function: Changes in lung function were observed in a large number of workplace studies at average concentrations of 1 µl/m³ and above; however, these changes were probably caused by peak exposures of 20 µl/m³ or much higher concentrations. In a longitudinal study covering a period of 17 years in total, 251 workers in PU production were exposed to concentrations of 0.3 to 1.05 µl/m³. The annual decreases in the FEV_1 and FVC were 35 ml and 30 ml, respectively, and were in the range of the values of the control group. The decreases in the lung function parameters correlated with the smoking behaviour and with the body weights of the workers, but not with exposure to TDI (Clark et al. 2003). According to the findings obtained in the study, lung function is not expected to be impaired after exposure to a concentration of 1 µl/m³.

Benchmark calculation: A LOAEC of 50 µl/m³ for chronic and necrotic rhinitis was derived from 2-year inhalation studies in rats and mice. A benchmark concentration cannot be calculated because chronic rhinitis of varying severity was observed in all mice even at the lowest concentration. A benchmark calculation for chronic rhinitis of severity 1 to 3 yielded $BMDL_{05}$ between 3 and 14 µl/m³ for female rats, which were found to be more susceptible than the males. The logistic model was determined to be the best model (lowest AIC value); the p value is 0.97. A BMD_{05} of 13 µl/m³ and a $BMDL_{05}$ of 10 µl/m³ were derived by applying this model. A concentration of 2 µl/m³ is calculated for TDI on the basis of the $BMDL_{05}$ of 10 µl/m³ and taking into consideration the extrapolation from animals to humans (1:3) and the preferred value approach. However, it has to be borne in mind that mice are more sensitive to these effects than rats.

A MAK value of 1 µl/m³ has been established for TDI on the basis of the benchmark calculation for chronic rhinitis in female rats and the observations of effects at the workplace and from volunteer studies.

Peak limitation. Sensory irritation is the decisive end point for the derivation of the MAK value; therefore, TDI has been classified in Peak Limitation Category I. TDI is a severely sensitizing substance; as a result, also in analogy to other diisocyanates, an excursion factor of 1 has been established. In field studies, effects on the airways were observed at the shift average value of about 5 µl/m³; however, peak exposures are considered to be responsible for the irritation that occurred. A momentary value of 5 µl/m³ has been established for TDI to prevent exposure even for very short periods of time to high concentrations that cannot be determined as a 15-minute mean.

Prenatal toxicity. In a prenatal developmental toxicity study in Sprague Dawley rats exposed to TDI by inhalation from days 6 to 15 of gestation, the incidence of incomplete ossification of the 5th cervical vertebra was increased at a concentration of 500 µl/m³ with concurrent maternal toxicity. The NOAEC for developmental and maternal toxicity was 100 µl/m³ for TDI (Greim 1999; Tyl et al. 1999 b). In a 2-generation study in Sprague Dawley rats with inhalation exposure, perinatal toxicity was not observed up to the highest TDI concentration tested of 300 µl/m³. Rhinitis was seen in the F0 and F1 adults at the lowest TDI concentration tested of 20 µl/m³ and above. The NOAEC for perinatal toxicity was 300 µl/m³ for TDI (Greim 1999; Tyl et al. 1999 a).

Taking into account the increased respiratory volume (1:2) and based on the NOAEC for TDI of 100 µl/m³ for developmental toxicity and of 300 µl/m³ for perinatal toxicity, a concentration of 50 and 150 µl/m³, respectively, is obtained and thus a margin of 50 and 150, respectively, to the MAK value for TDI of 1 µl/m³.

Toluene diisocyanates have been classified in Pregnancy Risk Group C because the margin between the NOAEC for developmental toxicity and the MAK value is sufficiently large, teratogenicity was not observed and the incomplete ossification of the 5th vertebra is regarded as a marginal and non-specific effect resulting from maternal toxicity.

Carcinogenicity. Reliable evidence of carcinogenicity in humans cannot be derived from the three available cohort studies. After inhalation exposure to TDI, no substance-induced increases in tumour incidences were observed in rats or mice. Oral exposure induced fibromas, fibrosarcomas, pancreatic tumours and neoplastic nodules in the liver in rats and haemangiosarcomas and liver tumours in mice. TDA is considered to be responsible for these tumours because they were found also after exposure to 2,4-TDA and TDA accounts for about 5% of the products formed after oral

absorption of TDI. In addition, the authors of the study reported that 10% to 23% of the TDI had already been broken down by the residual moisture in the corn oil and storage, leading to the formation of unidentified reaction products and polyurea (Dieter et al. 1990; NTP 1986). The carcinogenic risk associated with TDI therefore depends greatly on the amount of TDA formed in vivo (see Table 9).

Tab. 9 Comparison of the formation of TDA in rats after oral administration and inhalation exposure to TDI (according to Timchalk et al. 1994)

TDI	µg eq TDA overall/animal	TDA (µg eq/kg body weight) (body weight: 0.2 kg)
inhalation		
rat: 2 ml/m³, 4 hours (60 mg/kg body weight oral = 13 717 µg eq TDI; 2 ml/m³, 4 hours = 899 µg eq TDI, 2 ml/m³ = 4 mg/kg body weight)	0.26	1.3
1 mg TDI/kg body weight ≙ 0.325 µg eq TDA/kg body weight		
oral		
rat: 60 mg/kg body weight	15	75
1 mg TDI/kg body weight ≙ 1.25 µg eq TDA/kg body weight (3.8 times as much as after inhalation)		

After TDA was administered to rats in an oral dose of 3 mg/kg body weight, about the same amount of TDA was excreted as after oral administration of a TDI dose of 60 mg/kg body weight (Timchalk et al. 1994). Therefore, TDA makes up about 5% of the products formed from TDI. Inhalation exposure of rats to TDI for 4 hours in a concentration of 2 ml/m³ corresponds to an oral TDI dose of 4 mg/kg body weight; each rat excretes 0.26 µg of free and acetylated eq TDA. Table 9 shows that 3.8 times as much TDA is formed after oral administration as after inhalation exposure and thus 1.3% of TDI is metabolized to TDA after inhalation. Assuming that this ratio applies also to humans, a TDI dose of 1 µg/kg body weight would be absorbed after exposure to TDI at the level of the MAK value of 7 µg/m³ (1 µl/m³) and after 100% absorption; this corresponds to a TDA dose of 13 ng/kg body weight. After exposure to TDI at the carcinogenic dose of 60 mg/kg body weight, 5% TDA is formed, which corresponds to a TDA dose of 3 mg/kg body weight. The amount of TDA formed after exposure at the level of the MAK value is thus lower by a factor of 230 770 than after administration of the carcinogenic dose of 60 mg/kg body weight.

TDI has not been classified as a carcinogen because negative results were obtained in the inhalation study in rats and mice, clastogenic effects were not detected in vivo after inhalation and because it has been reliably estimated that only small amounts of TDA form from TDI after exposure at the level of the MAK value of 1 µl/m³. In addition, TDI doses like those observed in animal studies after oral exposure are not likely to occur at the workplace because of irritation.

Germ cell mutagenicity. In vitro mutations were induced in Salmonella typhimurium and in mouse lymphoma cells; they are attributed to the genotoxic metabolite 2,4-TDA. Likewise, the induction of sister chromatid exchange and chromosomal aberrations in CHO cells is attributed to 2,4-TDA.

Valid studies in workers with previous exposure to TDI did not yield evidence of DNA strand breaks in the lymphocytes. No DNA adducts, UDS or increases in micronuclei were detected in animals. Mutations in the SLRL test and reciprocal translocations were induced in Drosophila after administration via the diet. Studies of mutagenic effects in mammals are not available.

The clastogenic effects that were demonstrated for TDI in vitro were not detected in vivo. Mutagenicity data for TDI are not available; likewise, 2,4-TDA was not clastogenic in vivo, but mutagenic in the liver. Relevant mutagenic effects are not expected because only about 1% TDA was formed from the TDI dose that was taken up by inhalation. Therefore, TDI has not been classified in any of the categories for germ cell mutagens.

Absorption through the skin. Dermal LD_{50} values ≥ 10 000 mg/kg body weight suggest low acute dermal toxicity of TDI overall. Long-term studies of systemic toxicity after repeated dermal application are not available.

A recent animal study provides evidence of low overall absorption of TDI through the skin. The high reactivity of TDI and its affinity to structural components of the skin seem to considerably reduce the systemic availability of TDI and its active metabolite TDA. On the basis of the study findings, exposure of a surface area of skin of 2000 cm^2 (corresponding approximately to the area of both hands and forearms) to undiluted TDI for 1 hour would lead to the absorption of a TDI dose of 116 mg or 1.66 mg/kg body weight. Another animal study showed that after dermal exposure to TDI, about 0.3% of the dose was metabolized to TDA and excreted renally. Assuming similar conditions for humans, the exposure scenario described above would thus lead to a TDA dose of 5.0 µg/kg body weight. As described in Section 5.6.2.2, oral administration of TDI induced carcinogenic effects in an animal study at a corresponding TDA dose of 3 mg/kg body weight. This dose is 600 times as high as that in the dermal exposure scenario used for the evaluation.

The most important end point for the evaluation of possible dermal toxicity induced by TDI is skin sensitization, a property which has been unequivocally demonstrated for TDI. For this reason alone, skin contact is to be avoided. Currently, there is no reliable evidence that dermal contact alone induces systemically mediated respiratory allergies or other systemic effects. Therefore, additional labelling of the substance is not required and TDI has not been designated with an "H" (for substances which can be absorbed through the skin in toxicologically relevant amounts).

Sensitization. Although there are only relatively few case reports of dermatitis patients who reacted to TDI in patch tests, there is sufficient evidence of sensitizing effects induced by TDI (Hartwig 2015). Therefore, 2,4-TDI and 2,6-TDI have been designated with "Sh" (for substances which cause sensitization of the skin). Designation with "Sa" (for substances which cause sensitization of the airways) has been retained because of the clear sensitizing effects of TDI on the airways in humans. From the standpoint of occupational medicine, the most critical effects induced by the diisocyanates, which includes TDI, are the sensitizing effects on the airways. However, high or prolonged dermal exposure may also induce or contribute to respiratory tract sensitization. The workplace studies that are currently available do not allow the derivation of a concentration in the air below which sensitization of the airways is not induced.

Notes

Competing interests

The established rules and measures of the Commission to avoid conflicts of interest (www.dfg.de/mak/conflicts_interest) ensure that the content and conclusions of the publication are strictly science-based.

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