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A physiologically-based kinetic (PBK) model for work-related diisocyanate exposure: Relevance for the design and reporting of biomonitoring studies

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ABSTRACT

Diisocyanates are highly reactive substances and known causes of occupational asthma. Exposure occurs mainly in the occupational setting and can be assessed through biomonitoring which accounts for inhalation and dermal exposure and potential effects of protective equipment. However the interpretation of biomonitoring data can be challenging for chemicals with complex kinetic behavior and multiple exposure routes, as is the case for diisocyanates. To better understand the relation between external exposure and urinary concentrations of metabolites of diisocyanates, we developed a physiologically based kinetic (PBK) model for methylene bisphenyl isocyanate (MDI) and toluene di-isocyanate (TDI). The PBK model covers both inhalation and dermal exposure, and can be used to estimate biomarker levels after either single or chronic exposures. Key parameters such as absorption and elimination rates of diisocyanates were based on results from human controlled exposure studies. A global sensitivity analysis was performed on model predictions after assigning distributions reflecting a mixture of parameter uncertainty and population variability.

Although model-based predictions of urinary concentrations of the degradation products of MDI and TDI for longer-term exposure scenarios compared relatively well to empirical results for a limited set of biomonitoring studies in the peer-reviewed literature, validation of model predictions was difficult because of the many uncertainties regarding the precise exposure scenarios that were used. Sensitivity analyses indicated that parameters with a relatively large impact on model estimates included the fraction of diisocyanates absorbed and the binding rate of diisocyanates to albumin relative to other macro molecules.We additionally investigated the effects of timing of exposure and intermittent urination, and found that both had a considerable impact on estimated urinary biomarker levels. This suggests that these factors should be taken into account when interpreting biomonitoring data and included in the standard reporting of isocyanate biomonitoring studies.

1. Introduction

Diisocyanates are a group of highly reactive substances with two isocyanate functional groups. Diisocyanates are potent skin and respiratory tract sensitizers and a leading cause of occupational asthma in many industrial countries (Lockey et al. 2015; Malo and Chan-Yeung 2009). Exposure occurs mostly in occupational settings where these chemicals are widely used (RAC 2020a). Measuring diisocyanate exposure is indispensable for compliance with exposure limits and accurate risk assessment. However, measuring inhalation exposure to diisocyanates is technically challenging due to their reactivity (Health Council of the Netherlands 2018). In addition, air sampling does not provide information on dermal exposure. There is evidence that dermal exposure to diisocyanates plays a role in the development of respiratory sensitization and occupational asthma (Bello et al. 2007; Redlich 2010). Air measurements, typically performed outside the respiratory protective equipment (RPE) do not take into account the efficacy of RPE that is often used (McNally et al. 2012). However diisocyanates have complex kinetic behavior which hampers interpretation of biomonitoring data. From controlled human exposure studies after single diisocyanate

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exposures (Skarping, Brorson, and Sangö 1991; Brorson, Skarping, and Sango 1991; Budnik et al. 2011) we know that diisocyanates generally have a short half-life. As such, the interpretation of biomonitoring data is influenced by recent temporal factors such as the exposure scenario on that day (i.e. moment of exposure, exposure duration and exposure route) as well as subject behavior (i.e. use of PPE, moment of urination). Biomonitoring studies in chronically exposed workers indicate possible accumulation as elevated urinary amines in samples taken before the work shift on Monday morning have been reported (Tinnerberg et al. 2014; Geens et al. 2012). It has been hypothesized that this is due to formation of albumin adducts which circulate in the blood before being degraded and excreted (Health Council of the Netherlands 2018; ATSDR 2018). This is referred to as a two phase urinary elimination pattern, with the first phase being related to the more recent exposure and a half-life over several hours, and the second much slower one probably related to the degradation of TDI and MDI conjugates (Lind et al. 1996).

Here we aimed to give quantitative insight on how urinary biomarkers of diisocyanate exposure are affected by several factors - including potential dermal exposure, accumulation in chronically exposed workers and differences between diisocyanates in elimination kinetics. For these purposes physiologically based kinetic (PBK) models can be applied as they are known to be useful tools in data analysis to interpret the relation between external and internal exposures (Hoer et al. 2020). PBK models use differential equations to estimate the concentration of a chemical or its metabolites in urine or blood. A PBK model can extrapolate across exposure scenarios (thereby reducing the need for data on specific exposure scenarios), and help understand which parameters are most influential for kinetic and dynamic behavior of the chemical in question.

To that end we developed a PBK model for diisocyanates, focusing on two of the most commonly used isocyanates: 4,4′-methylene diphenyl diisocyanate (MDI) and toluene diisocyanate (TDI). To our knowledge there are two published PBK models available for (degradation products of) diisocyanates. Luu, Hutter, and Bushar (1998) developed a model to estimate exposure to 2,4-toluenediamine (TDA; a degradation product of TDI) as released from the degradation of the polyester urethane foam used in silicone breast implants. The other PBK model was developed to model exposure to 4,4-methylenedianiline (MDA; a degradation product of MDI) from the potting materials of hemodialyzers (Luu and Hutter 2000). Until now there is no PBK model available for diisocyanates that estimates biomarker levels in urine after chronic exposure to TDI or MDI via both inhalation and dermal exposure, and includes accumulation of metabolite levels due to the formation of plasma adducts. For deriving diisocyanate biological limit values based on external health based limit values (i.e. occupational exposure limits (OEL)), current practice is to derive correlation formulae based on occupational studies that report both air and urine measurements. We hypothesized that it would be useful to develop a PBK model to better understand and characterize how factors such as the exposure scenario and subject behavior have an effect on the presumed external – internal exposure relation.

2. Methods

2.1. PBK model

2.1.1. Model structure

Our proposed diisocyanate PBK model (Fig. 1) includes the following main compartments: lungs, skin, gut lumen, kidney, arterial blood, venous blood, and all other ("remaining") parts of the body. Because diisocyanates are known to reside in the lungs for some time before they are taken up in the blood circulation (ATSDR 2018), we distinguish between a combined alveolar air, epithelial lining fluid and lung tissue ("interstitial") compartment and a ("vascular") compartment that is directly connected to the systemic circulation. Most diisocyanates are expected to bind rapidly to albumin and other macromolecules and we therefore distinguish between unbound ("[ub]"), albumin-bound ("[alb]"), and (other) macro-molecule-bound ("[mcr]") fractions within each of the compartments.

The model allows for exposure to diisocyanates through inhalation and through skin absorption. Following exposure, transport and

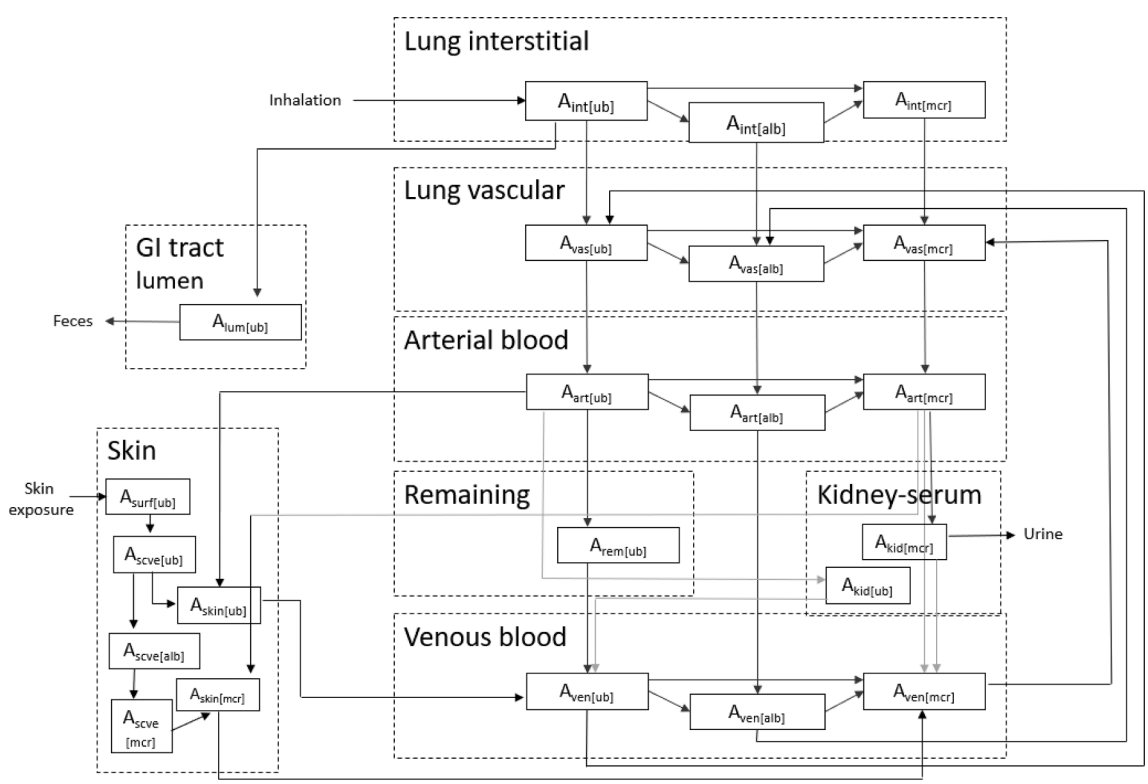

Fig. 1. Schematic picture of physiological based kinetic model for diisocyanates (MDI and TDI).

distribution of diisocyanates across the different compartments is assumed to be driven by blood flow rates. Elimination of diisocyanates and their conjugated forms is included in the PBK model via the urine or feces. Urinary excretion of absorbed isocyanates is expected to take place following degradation of albumin. We accommodate the relatively long(er) retention of albumin-bound diisocyanates by modelling excretion only from the macro-molecule-bound fraction and using a relatively slow transfer (elimination) from the albumin-bound fraction into this compartment.

2.1.2. Differential equations

For each of the compartments shown in Fig. 1, the amount (in mg) and concentration (in mg/L) of diisocyanates in each state (i.e. unbound, albumin-bound, or macro-molecule bound) is indicated by $A_{compartment[state]}$ and $C_{compartment[state]}$ respectively, where the concentration is calculated by dividing the amount by the compartment volume.

A detailed description of the compartments involved in the uptake and urinary excretion of diisocyanates and the (initial) distribution of diisocyanates across different fractions is provided below. A description of other compartments, that are mainly involved only in transport of diisocyanates across the body, is provided in the appendix (section A).

Lungs.

The amounts of unbound, albumin-bound, and (other) macro-molecule-bound diisocyanates in the interstitial lung compartment are described by the following differential equations (Eq. (1) – (3)):

$$dt(A_{int[ub]}) = Q_{br}*F_{abs}*C_{air} - k_{alb}*A_{int[ub]} - k_{mcr}*A_{int[ub]} - k_{vas}*A_{int[ub]} \quad (1)$$

$$dt(A_{int[alb]}) = k_{alb}*A_{int[ub]} - k_{elim}*A_{int[alb]} - k_{vas}*A_{int[alb]} \quad (2)$$

$$dt(A_{int[mcr]}) = k_{mcr}*A_{int[ub]} + k_{elim}*A_{int[alb]} - k_{vas}*A_{int[mcr]} \quad (3)$$

where Q_{br} is the breathing rate in L/h, F_{abs} is the fraction absorbed (the non-absorbed fraction is excreted through the gastrointestinal tract), C_{air} is the inhalation exposure in mg/L, k_{alb} and k_{mcr} are the binding rate constants (unit h^{-1}) for binding of unbound diisocyanates to albumin and other macromolecules, k_{vas} is the transfer rate constant (unit h^{-1}) for transfer from the interstitial to the vascular lung compartment, and k_{elim} is the elimination rate constant (unit h^{-1}) for the degradation of albumin into (smaller) macromolecules.

The amounts of unbound, albumin-bound, and (other) macro-molecule-bound diisocyanates in the vascular lung compartment are described by the following differential equations (Eq. (4) – (6)):

$$dt(A_{vas[ub]}) = k_{vas}*A_{int[ub]} - k_{alb}*A_{vas[ub]} - k_{mcr}*A_{vas[ub]} + Q_c*(C_{ven[ub]} - C_{vas[ub]}) \quad (4)$$

$$dt(A_{vas[alb]}) = k_{vas}*A_{int[ub]} + k_{alb}*A_{vas[ub]} - k_{elim}*A_{vas[ub]} + Q_c*(C_{ven[alb]} - C_{vas[alb]}) \quad (5)$$

$$dt(A_{vas[mcr]}) = k_{vas}*A_{int[mcr]} + k_{mcr}*A_{vas[ub]} + k_{elim}*A_{vas[ub]} + Q_c*(C_{ven[mcr]} - C_{vas[mcr]}) \quad (6)$$

where Q_c is the cardiac output (in L/h).

Dermal exposure.

The amounts of (unbound) diisocyanates on the surface of the skin and stratum corneum including viable epidermis (scve) are described by the following equations (Eq. 7–10):

$$dt(A_{surf[ub]}) = SA_{derm}*D_{derm[ub]} - Q_{scve}*(C_{surf[ub]} - C_{scve[ub]}) - k_{evap}*A_{surf[ub]} \quad (7)$$

$$dt(A_{scve[ub]}) = Q_{scve}*(C_{surf[ub]} - C_{scve[ub]}) - K_{alb[scve]}*A_{scve[ub]} - P_{bld[scve]}*Q_{scve}*C_{scve[ub]} \quad (8)$$

$$dt(A_{scve[alb]}) = K_{alb[scve]}*A_{scve[ub]} - K_{elim}*A_{scve[alb]} \quad (9)$$

$$dt(A_{scve[mcr]}) = K_{elim}*A_{scve[alb]} - P_{bld[scve]}*Q_{scve}*C_{scve[mcr]} \quad (10)$$

with SA_{derm} the exposed surface area of the skin (in cm^2), D_{derm} the dose applied to the skin (in mg/cm^2/hour), Q_{scve} the fat-content adjusted stratum corneum permeability coefficient transferred into a clearance value (in L/h), k_{evap} the dermal evaporation rate (unit h^{-1}; see below), $K_{alb[scve]}$ the scve-specific binding rate constant of binding to albumin (and perhaps other macro-molecules), and $P_{bld[scve]}$ the stratum corneum and viable epidermis to blood partition coefficient. The formula used to calculate the dermal evaporation rate (k_{evap}) is loosely based on (ECHA 2009) and includes the molecular weight (MW) and vapor pressure (VP, unit Pa) of the diisocyanate:

$$k_{evap} = c_{evap} \times \frac{MW \times 8.7 \times VP \times SA_{derm}}{8.314*303.0*10}$$

Based on the study by Hamada (2018), the constant c_{evap} was fixed to achieve an approximate 50% evaporation rate for MDI (Eq. (11) – (12)).

The amounts of diisocyanates in the different fractions in the skin compartment are described by the following equations:

$$dt(A_{skn[ub]}) = Q_{skn}*(C_{art[ub]} - C_{skn[ub]}) + P_{bld[scve]}*Q_{scve}*C_{scve[ub]} \quad (11)$$

$$dt(A_{skn[mcr]}) = -Q_{skn}*C_{skn[mcr]} + P_{bld[scve]}*Q_{scve}*Q_{scve[mcr]} \quad (12)$$

where Q_{skn} is the blood flow to and from the skin compartment (in L/h). Note that we assume that albumin-bound diisocyanates leave the skin compartment only after degradation of the albumin (elimination).

Urinary excretion.

Usually urine samples are treated with bases or acids to hydrolyze the diisocyanate-protein or diamine-protein conjugates and acetylated diamines, resulting in the formation of free diamine (Sennbro et al. 2004). With our model we estimate the levels of conjugates in urine (which are excreted as low-molecular weight conjugates), and that are ultimately converted to amines. These are TDA and MDA, for TDI and MDI respectively.

The amount of diisocyanates excreted in urine is determined by the Q_{gfr} in L/h and the amount of diisocyanate in the macromolecule and metabolite compartments of the kidneys, and is converted to milligrams of the amine metabolite using the ratio of molecular weights:

$$dt(A_{kid[mcr]}) = Q_{kid}*(C_{art[mcr]} - C_{kid[mcr]}) - Q_{gfr}*C_{art[mcr]} \quad (13)$$

$$dt(A_{kid[ub]}) = Q_{kid}*(C_{art[ub]} - C_{kid[ub]}) \quad (14)$$

$$dt(A_{uri[amine]}) = \frac{MW_{amine}}{MW_{parent}}*Q_{gfr}*(C_{kid[mcr]} \quad (15)$$

where Q_{gfr} is the glomerular filtration rate (in L/h).

The urinary concentration of diisocyanate amines is then calculated using the following formula (Eq. (16)) for the urine production rate:

$$dt(V_{uri}) = Q_{upr} \quad (16)$$

where Q_{upr} is the bodyweight specific urinary production rate (unit L/h). We assume at each void that the bladder is fully emptying.

When reporting we use the creatinine adjusted concentrations by dividing the estimated amines concentrations by the urinary creatinine concentration (parameter C_{creat}).

2.1.3. Model parameters

A description of the various parameters and how these are derived or calculated, can be found in Table 1. Table 2 includes an overview of the hyperparameters that are used for the calculation or derivation of the parameters from Table 1. Information from various (clinical) studies was used to determine the central estimates, ranges and uncertainty of the physiological parameters in the PBK model. Some parameter values were not readily available in the literature and were therefore calibrated

Table 1
Description of parameters used for differential equations and corresponding formulas.

Parameter name	Description	Formulae
K_{vas}	Transfer rate constant for transfer from the interstitial to the vascular lung compartment (h^{-1})	$Log(2)/T_{vas}$
K_{alb}	Binding rate constants for binding of unbound diisocyanates to albumin (h^{-1})	$Falb*Log(2)/T_{albmcr}$
K_{mcr}	Binding rate constants for binding of unbound diisocyanates to macromolecules (h^{-1})	$(1-Falb)*(Log(2)/T_{albmcr})$
K_{elim}	Elimination rate constant for the degradation of albumin into (smaller) macromolecules) (h^{-1})	$Log(2)/K_{elim}$
k_{hydro}	Rate constant for hydrolysis of unbound diisocyanates (h-1)	$Log(2)/T_{hydro}$
K_{feces}	Transfer rate constant from the gut lumen to the feces	$Log(2)/T_{feces}$
$K_{alb[scve]}$	Binding rate constant of binding to albumin in the skin	$Log(2)/T_{alb[scve]}$
$P_{bld[scve]}$	Skin barrier (stratum corneum + viable epidermis) to blood partition coefficient	$(1-Ffat_{epi} + Ffat_{epi}*10^{logp})/(1-Ffat_{bld} + Ffat_{bld}*10^{logp})$
Q_{scve}	Fat-content adjusted stratum corneum permeability coefficient transferred into a clearance value (L/h)	$10^{0.74*logp - 0.006*MW - 2.8} * SA_{derm}/1000$
Q_{upr}	Urine production rate (L/h)	$UPR*BW$
Q_{gut}	Blood flow to the gut (L/h)	$QF_{gut}*Qc$
Q_{liv}	Blood flow to the liver (L/h)	$QF_{liv}*Qc$
Q_{skn}	Blood flow to the skin (L/h)	$QF_{skn}*Qc$

using data obtained from human experimental exposure studies (Skarping, Brorson, and Sangö 1991; Brorson, Skarping, and Sango 1991; Budnik et al. 2011). For example, the estimate of the fraction absorbed ("F_{abs}") was based on experimental data suggesting that approximately 20% of diisocyanate is absorbed and excreted in urine. Another example is the half-life transfer from the lung interstitial to the lung vascular compartment (T_{vas}, hr), which is calibrated such that after about 6 h most of TDI has been excreted, based on the studies by Brorson, Skarping, and Sangö (1991), Budnik et al. (2011). MDI is excreted more slowly (Budnik et al. 2011) and therefore the T_{vas} values for TDI and MDI differ. T_{vas} is subsequently converted to a transfer rate constant (K_{vas}, unit h^{-1}) (Table 1). A more detailed description of the controlled human experimental exposure studies can be found in the Appendix (section B).

2.1.4. Exposure scenarios

2.1.4.1. Short-term and chronic inhalation exposure. As several model parameters were based on information gathered from human experimental exposure studies, we first confirmed whether our model was able to mimic the time course of urinary excretion in these studies. Most of these studies used single, relatively short-term, exposures, and we therefore refer to these as "short-term exposure" scenarios. However, as our main interest was in modeling urinary excretion after occupational exposure, our main results are based on a scenario where exposure occurred continuously for 8 h a day (from 9:00 to 17:00) on 5 days a week, for a total of 8 weeks. We refer to this scenario as the "chronic exposure" scenario. For this scenario, workers were assumed to urinate, at 8:00, 13:00, 18:00, and 23:00. Reported urinary concentrations relate to the bladder content just prior to the urination at 18:00.

2.1.4.2. Contribution of dermal exposure. For combined inhalation and dermal exposure, we used an exposure scenario that was based on an

Table 2
Hyperparameters for PBK model. N: normal (distribution), T N: truncated normal (distribution).

Model parameter	Description	Default value	Uncertainty distribution	Reference
Chemical specific				
MW	Molecular weight of the parent compound	174.16 (TDI) 250.25 (MDI)	–	
MW_{met}	Molecular weight of the amine	122.17 (TDA) 198.26 (MDA)	–	
LogP	LogP	3.74 (TDI) 5.22 (MDI)	–	(PubChem 2022) (USEPA 2011)
VP	Vapor pressure (Pa)	2.7 (TDI) 4.9×10^{-4} (MDI)	–	(Health Council of the Netherlands, 2018)
F_{abs}	Fraction absorbed	0.2	U [0.1–0.3]	Based on the controlled human exposure study of Budnik et al. (2011) for MDI and TDI, for TDI only the study by Brorson, Skarping, and Sangö (1991), and for MDI the animal study by Gledhill et al. (2005).
T_{albmcr}	Half-life of binding to albumin and other macromolecules (hr)	TDI: 0.167 MDI: 1.5	TDI: U [0.033 – 0.300] MDI: U [0.3 – 2.7]	
F_{alb}	Proportion bound to albumin (%)	0.2	U [0.04 – 0.36]	
T_{vas}	Half-life transfer from lung interstitial to lung vascular (hr)	TDI: 0.083 MDI: 14	TDI: U [0.017 – 0.150] MDI: U [2.8 – 25.2]	
Individual				
BW	Body weight (kg)	85	T N (85, 8.5) [68 – 102]	
(volume) fractions				
VF_{bld}	Volume fraction blood	0.073	T N (0.073,0.007) [0.058 – 0.088]	The volumes of compartments were based on the values reported for adult males (Valentin and Streffer 2002)
VF_{lun}	Volume fraction lung	0.015	T N (0.015, 0.002) [0.012 – 0.018]	
VF_{skn}	Volume fraction skin	0.045	T N (0.045, 0.005) [0.036 – 0.054]	
VF_{gut}	Volume fraction gut	0.016	T N (0.016,0.002) [0.013 – 0.019]	
V_{lumen}	Volume gut lumen	0.9	T N (0.90, 0.090) [0.72 – 1.08]	
V_{kidney}	Volume kidney	0.004	T N (0.004, 0.0004) [0.003, 0.005]	
F_{hem}	% hematocrit	0.45	T N (0.45, 0.045) [0.36 – 0.54]	
Blood flows				
QF_{skn}	Fractional blood flow to skin	0.05	T N (0.050, 0.003) [0.045 – 0.055]	The blood flows were based on the values reported for adult males (Valentin and Streffer 2002)
QF_{gut}	Fractional blood flow to gut	0.15	T N (0.150, 0.08)	

(continued on next page)

Table 2 (*continued*)

Model parameter	Description	Default value	Uncertainty distribution	Reference
			[0.135 – 0.165]	
Flow rates				
Q_{br}	Breathing rate (L/h)	1000	N (1000, 100) [800,1200]	(HERAG 2007)
Q_c	Cardiac output (L/h)	390	T N (390, 19.5) [351 – 430]	(Valentin and Streffer 2002)
Q_{gfr}	Glomerular filtration rate (L/h)	5.5	N (5.5, 0.7) [4.0,7.0]	(Redal-Baigorri, Rasmussen, and Heaf 2014)
T_{feces}	Half-life transfer lumen to feces (/hr)	2.3	U [2.1 – 2.5]	
Excretion				
UPR	Urine production rate (L/kg/hr)	0.00125	T N (0.00125, 0.00006) [0.00113 – 0.00138]	
Ccreat	Creatinine in urine (g/L)	1.0	T N (1.0, 0.05) [0.8 – 2.0]	(Valentin and Streffer 2002)
T_{elim}	Half-life elimination from albumin (hr)	456	T N (456, 45.7) [364 – 547]	(Reed 1988)
Skin parameters				
SA_{derm}	Exposed surface area of skin (cm^2)	100	T N (100, 5) [90 – 110]	
H_{scve}	Height of skin barrier (cm)	0.012	T N (0.012, 0.001) [0.010 – 0.013]	
H_{surf}	Height of skin surface (cm)	0.010	T N (0.01, 0.0001) [0.009 – 0.011]	
Fat_{epi}	Fraction of fat in epidermis	0.02	T N (0.02, 0.001) [0.018 – 0.022]	
Fat_{bld}	Fraction of fat in blood	0.07	T N (0.07, 0.004) [0.063 – 0.077]	
$T_{alb[scve]}$	Half-life of binding to albumin in scve (hr)	$2.8*10^{-6}$	U [$5.6*10^{-7}$ – $5.0*10^{-6}$]	

observational study by Henriks-Eckerman et al. (2015). The authors provided information on MDI exposure levels for both inhalation and dermal exposure for workers in the construction and boat building industry. Cumulative dermal exposure was assessed using a strip-tape technique and was estimated to average between 0.16 and 1.38 μg/10 cm^2, depending on the exact site (on the lower arm) that was sampled, while inhalation exposure was estimated to be between 0.08 and 0.8 μg/m^3. Tasks involving MDI exposure in this study typically lasted between 15 and 60 min, with each task repeated 2–20 times per shift. Based on this information, we designed our dermal exposure scenario to consist of continuous exposure to 0.015 μg MDI/cm^2/hour for 4 h, equivalent to a cumulative exposure of 0.6 μg/10 cm^2. To investigate the (potential) impact of dermal exposures on total excreted urinary diisocyanate levels, we compared the output from our model for dermal exposure either with or without concurrent inhalation exposure to 0.4 μg/m^3 MDI.

2.1.5. Model comparison with published literature

To compare model predictions for chronic exposures with those reported in the literature we searched for reports from biomonitoring studies that included estimates of both external exposure and urinary concentrations in workers that were exposed to diisocyanates for a prolonged time-period. We collected information on urinary concentrations of diisocyanates, sampling times (i.e. pre-shift or post shift), potential dermal exposure, use of RPE, and task-based and/or time-weighted-average (TWA) exposures. Exposures below the limit of detection (LOD) were replaced by half the reported LOD. In case of task-based estimates of inhalation exposure only, we estimated TWA exposure under the assumption that there was no exposure outside the task.

For TDI we extracted five studies that reported post-shift urinary measurements (Sennbro et al. 2004; Maître et al. 1993; Geens et al. 2012; Austin 2007; Swierczyńska-Machura et al. 2015), and two studies that reported pre-sh ift urinary measurements (Geens et al. 2012; Tinnerberg et al. 2014) (Appendix section C). For MDI we extracted three studies that reported post-shift urinary measurements (Sennbro et al. 2006; Henriks-Eckerman et al. 2015; Bello et al. 2019), and one study that reported pre-shift urinary measurements (Tinnerberg et al. 2014) (Appendix D). An overview of estimated exposure levels and urinary diisocyanate concentrations for these studies is provided in the appendix (tables S1and S2).

2.2. Sensitivity analysis

Where applicable, we report 95% uncertainty intervals (95%UI) that reflect a mix of true parameter uncertainty and between-worker variability in morphological parameters. Uncertainty intervals were estimated by Monte-Carlo simulation, taking the empirical quantiles of 1,000 different outputs from the PBPK model, where each output was generated using parameters that were randomly drawn from the distributions presented in Table 2.

Based on the input parameter distributions shown in Table 1, we conducted a global sensitivity analysis (GSA) using an extended Fourier Amplitude Sensitivity Test (eFAST) (McNally et al., 2012). The scenario used for this analysis was the "long-term exposure" scenario (consisting of 8 weeks exposure for 8 h a day) with the focus on post-shift urinary concentrations on the last working-day at the end of this period.

Most biomonitoring studies provide little information on the timing of task-based exposures relative to the time of urine sample collection or on the frequency and timing of any intermediate urination, which could both contribute to increased exposure misclassification. We therefore investigated the sensitivity of estimated post-shift urinary diisocyanate concentrations for task-based exposures by varying the timing of exposure and the frequency and timing of intermediate urination moments.

2.3. Software

The PBK model was programmed in MCSim (version 6.2) and the R package PKSensi (version 1.2.3) was used to perform the global sensitivity analysis.

3. Results

3.1. Urinary biomarker levels following short-term exposure

Estimated urinary concentrations of TDA following a single inhalation exposure to 40 μg/m^3 of TDI for 4 h are shown in the appendix (Figure S1, Appendix section E). Excretion of urinary TDA peaked between 4 and 6 h after exposure started. Most of the absorbed dose was eliminated within 10 h after the end of exposure. This pattern is qualitatively similar to that described for human volunteers in the studies by Skarping, Brorson, and Sangö (1991), Brorson, Skarping, and Sango (1991), Budnik et al. (2011). In those controlled experimental studies, the peak of excretion occurred roughly 2 to 6 h after exposure had started, with most of the TDI excreted in urine within 24 h from the end of exposure. The estimated urinary concentration of MDA after a single inhalation exposure to 40 μg/m3 of MDI for 4 h is also shown in the

appendix (Figure S2, Appendix section E). Excretion of MDA peaked approximately 10 h after the start of exposure, with excretion continuing long after exposure ceased. This pattern is similar to that described for human volunteers exposed to MDI in the study by Budnik et al. (2011), where the authors reported that excretion peaked approximately 14 h after exposure and elimination was not complete after 24 h.

3.2. Urinary biomarker levels following chronic inhalation exposure

3.2.1. Comparison to literature

The exposure–response relation between air concentrations of TDI and estimated post-shift TDA urinary concentrations on the last workday of the 8 weeks exposure period is shown in Fig. 2A.

The figure includes results from published occupational studies, with the legend indicating whether the study reported use of respiratory protective equipment (REP) by at least some of the workers.

There was a linear relation between air exposure and urinary concentrations, but with significant uncertainty in estimated TDA levels that included a nearly 5-fold difference between upper and lower boundaries of the uncertainty interval. Results from published occupational studies are included in the plot, to allow informal comparison. Model predictions were higher than reported urinary concentrations for almost all studies, note however that reported values were based on average exposures and average urinary concentrations for groups of workers and that respiratory protective equipment had also been used in some of the studies. The exposure–response relation between air concentrations of MDI and estimated post-shift MDA urinary concentrations on the last workday of the 8 weeks exposure period is shown in Fig. 2B. As for TDI, results from occupational studies were included for informal comparison. From the results it appears that model predictions are lower compared to all three published studies. A more detailed comparison is provided in the Discussion.

3.2.2. Kinetics

The evolution of urinary concentrations of TDA over a week period after repeated exposure to 20 μg/m3 TDI for 8 h per day and for five days a week is provided in Fig. 3A. Levels of urinary TDA after the weekend on Monday morning before the shift were estimated to be approximately 1.5 μg TDA/gr creatinine. An overview of the time evolution of urinary concentrations of MDA over aweek period after repeated exposure to 1.25 μg/m^3 MDI for 8 h per day and for five days a week is provided in Fig. 3B. Levels of urinary MDA after the weekend on Monday morning before the shift and after the shift on Friday afternoon in week 8 were estimated to be approximately 0.18 and 0.7 μg MDA/gr creatinine respectively.

Model estimates for concentration of albumin adducts upon repeated exposures to TDI and MDI for 8 h per day and for five days a week is provided in Fig. 3C and 3D. From these figures it appears that adducts in blood accumulate over a long period and appear to stabilize after about 100 days for both MDI and TDI.

3.2.3. Impact of (short-term) dermal exposure to MDI

Our combined inhalation and dermal exposure scenario was based on results reported in the study by Henriks-Eckerman et al. (2015) and consisted of exposure to MDI in the air at a concentration of 0.4 μg/m^3 and a (dermal) deposition rate of 0.015 μg /cm^2/hour for 4 h.

When including both inhalation and dermal exposure (Fig. S5a, Appendix section F), MDA urinary levels were estimated to be 0.02 and 0.10 μg/gr creatinine, directly after exposure and 4 h later, respectively. Without inhalation exposure, MDA urinary levels were estimated to be 0.01 and 0.05 μg/gr creatinine, directly after exposure and 4 h later, respectively (Fig. S5b, Appendix section F). The relative contribution of dermal exposure to total urinary excretion within 72 h after exposure was approximately 68% and 8.8% of the total dermal dose had been excreted within 72 h.

3.3. Parameter sensitivity for a fixed exposure scenario

We performed a global sensitivity analysis to assess which parameters contributed most to the uncertainty in model predictions for the "chronic" inhalation exposure scenario. For both TDI and MDI, the uncertainty in "fraction absorbed" appeared to have the largest impact on overall uncertainty, accounting for approximately 40% of total uncertainty in urinary biomarker levels obtained just after the last shift (on Friday evening) in the last week of the simulated exposure period (Figs. 4 and 5). Other important contributors to overall uncertainty in predictions were the binding rate of TDI to albumin and other macro molecules and the body weight of subjects (which is often not recorded). For MDI, uncertainty in the rate of transfer for macro-molecule bound MDI from the "interstitial" to the "vascular" lung compartment was also important.

We also evaluated global sensitivity for an alternative timepoint. Figures S6 and S7 (Appendix section G) show the results for urinary biomarker levels obtained just before the first shift (on Monday morning) in the last week of the simulated exposure period. The results show a shift in importance for some parameters, with the proportion bound to albumin (F_{alb}) being relatively more important than for post-shift levels the following Friday.

3.3.1. Temporal factors in exposure and biomarker collection

The impact of differences in the timing of inhalation exposure relative to that for biomarker collection on estimated urinary concentrations is shown in Fig. 6. Results were based on a short-term exposure scenario in which workers were exposed to 7 μg TDI/m^3 for 4 h, but where

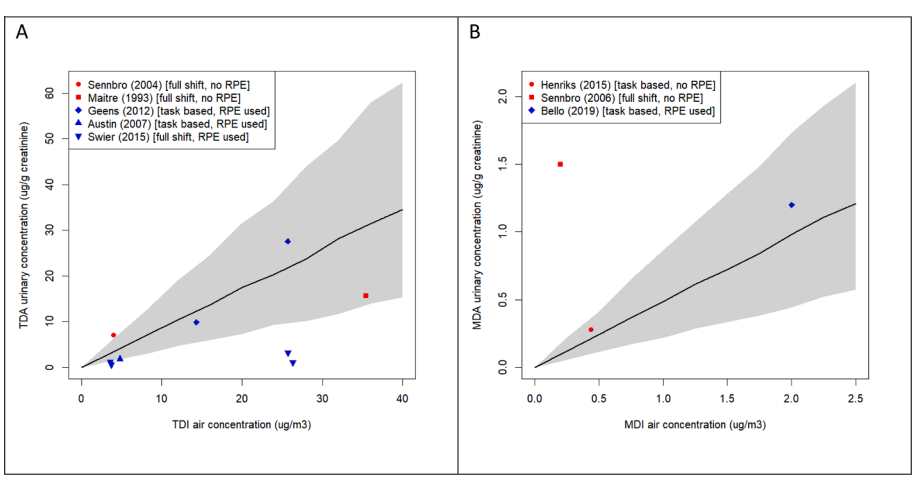

Fig. 2. Exposure-response curve for urinary concentrations at the post-shift urination on the last working day (A) Predicted urinary TDA concentrations (in μg/gr creatinine) following repeated occupational exposure. (B) Predicted urinary MDA concentrations (in μg/gr creatinine) following repeated occupational exposure. Exposure was assumed to occur on 5 days a week, for 8 h a day (from 9:00 to 17:00), for a period of 8 weeks, with daily urination at 8:00, 13:00, 18:00, and 23:00. The solid black lines are the estimated concentrations using the most likely ("default") values for all parameters, while the ribbon indicated the 95% uncertainty intervals generated from the uncertainty distributions.

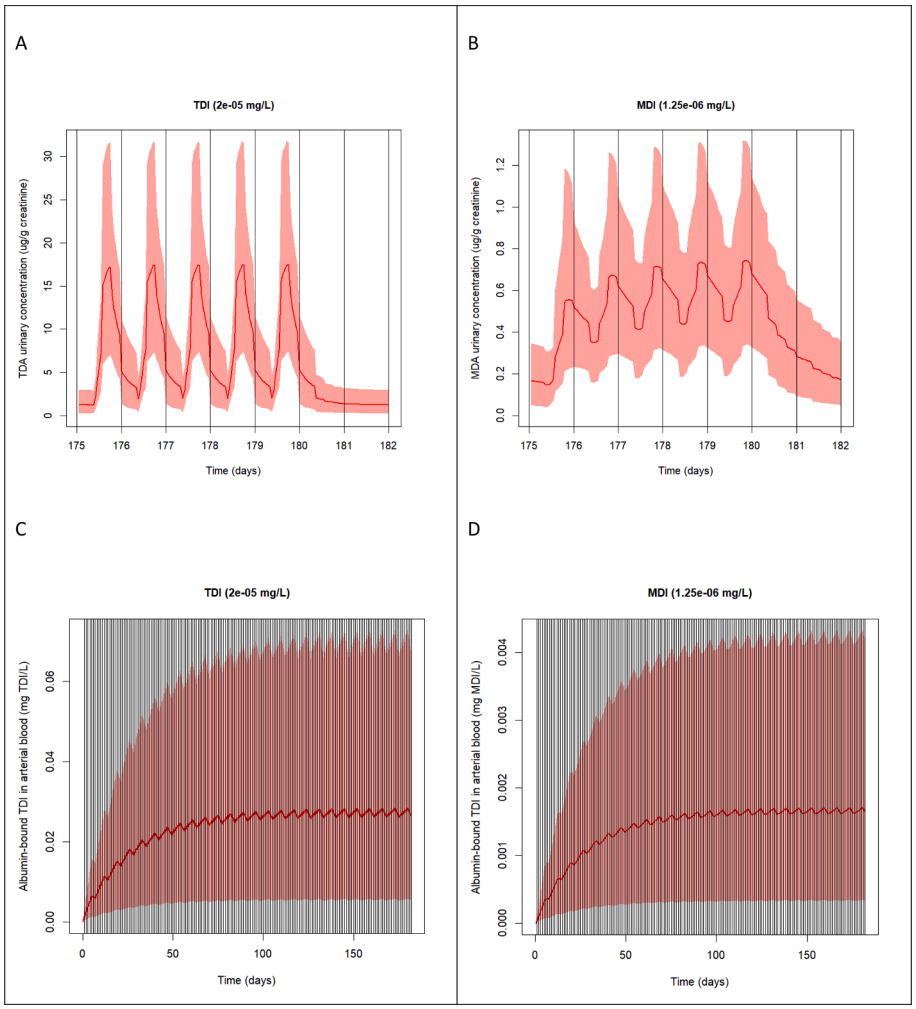

Fig. 3. Predicted urinary MDA concentrations (in μg/gr creatinine) following repeated occupational exposure. () Time evolution after exposure to 20 μg/m^3 TDI. (B) Time evolution after exposure to 1.25 μg/m^3 MDI. (C) Predicted albumin-bound TDI in arterial blood (in mg/L) following repeated occupational exposure to 20 μg/m^3 TDI. D) Predicted albumin-bound MDI in arterial blood (in mg/L) following repeated occupational exposure to 1.25 μg/m^3 MDI. Exposure was assumed to occur on 5 days a week, for 8 h a day (from 9:00 to 17:00), with daily urination at 8:00, 13:00, 18:00, and 23:00. The red ribbons show the 95%UI. Exposure was assumed to occur on 5 days a week, for 8 h a day (from 9:00 to 17:00), with daily urination at 8:00, 13:00, 18:00, and 23:00. The red ribbons show the 95%UI. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

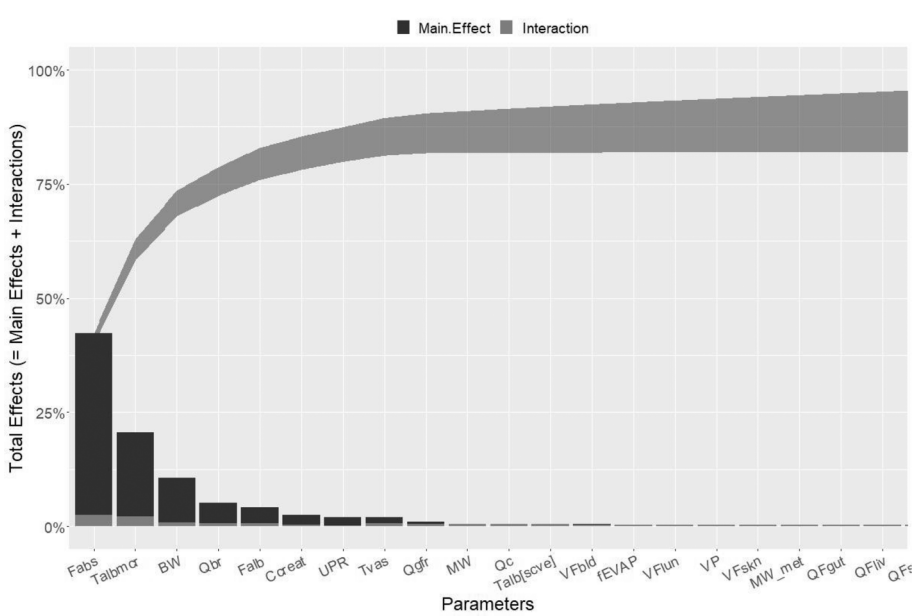

Fig. 4. Lowry plot of results from the eFAST sensitivity analysis for predicted urinary concentrations of TDA (in μg/gr creatinine) after exposure to 20 μg/m^3 TDI at the post-shift urination on the last working day. Exposure was assumed to occur on 5 days a week, for 8 h a day (from 9:00 to 17:00), for a period of 8 weeks, with daily urination at 8:00, 13:00, 18:00, and 23:00. The total effect is comprised of the main effects (black bar) and possible interactions with other parameters (grey bar) given as the proportion of variance in the output parameter. The grey ribbon indicates the cumulative proportion of variance explained due to main effects and parameter interactions.

exposure either started in the early morning at 9:00 ("early") or in the afternoon at 13:00 ("late"). Urination was at 8:00, 13:00, 18:00, and 23:00 h for both scenarios. Estimated TDA concentrations in urine obtained at 18:00 were approximately 2.7 μg/gr creatinine for the "early" scenario (Fig. 6a), and 2.6 μg/gr creatinine for the "late" scenario (Fig. 6b).

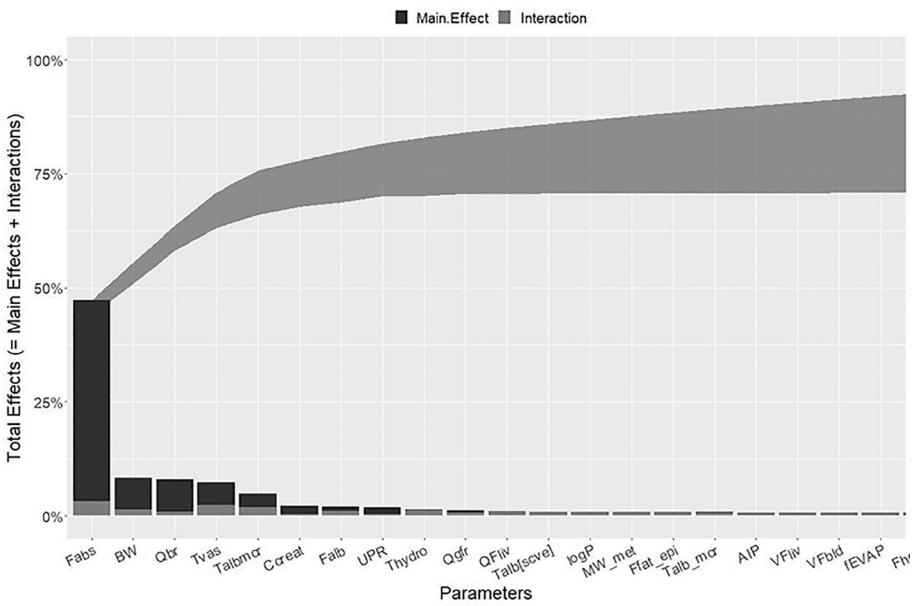

Fig. 5. Lowry plot of results from the eFAST sensitivity analysis for predicted urinary concentrations of MDA (in μg/gr creatinine) after exposure to 1.25 μg/m^3 MDI at the post-shift urination on the last working day. Exposure was assumed to occur on 5 days a week, for 8 h a day (from 9:00 to 17:00), for a period of 8 weeks, with daily urination at 8:00, 13:00, 18:00, and 23:00. The total effect is comprised of the main effects (black bar) and possible interactions with other parameters (grey bar) given as the proportion of variance in the output parameter. The grey ribbon indicates the cumulative proportion of variance explained due to main effects and parameter interactions.

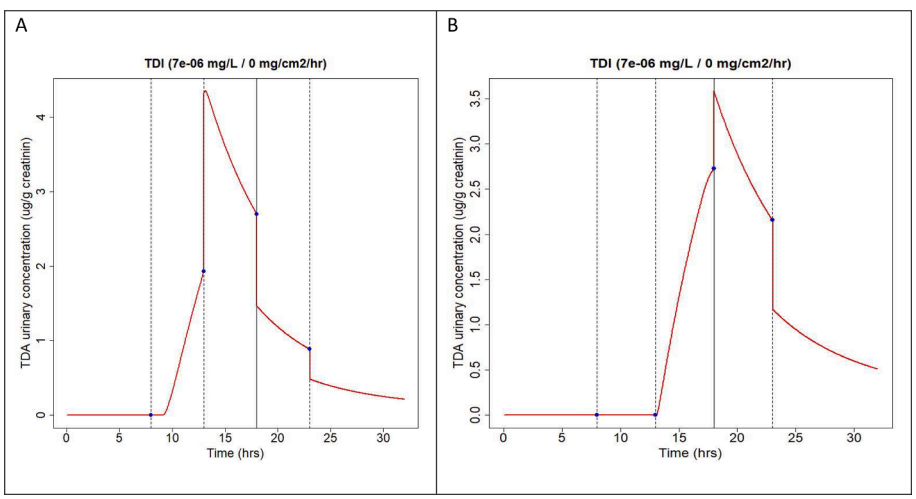

Fig. 6. Effect of the timing of short-term exposure to TDI (7 μg/m^3) on predicted urinary concentrations. For the early exposure scenario (A) exposure is from 9:00 to 13:00, with urination at 8:00, 13:00, 17:00, and 23:00. For the late exposure scenario (B) exposure is from 13:00 to 17:00, with the same urination moments. Urination moments are indicated by dotted lines with predicted urinary concentrations indicated by a blue dot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

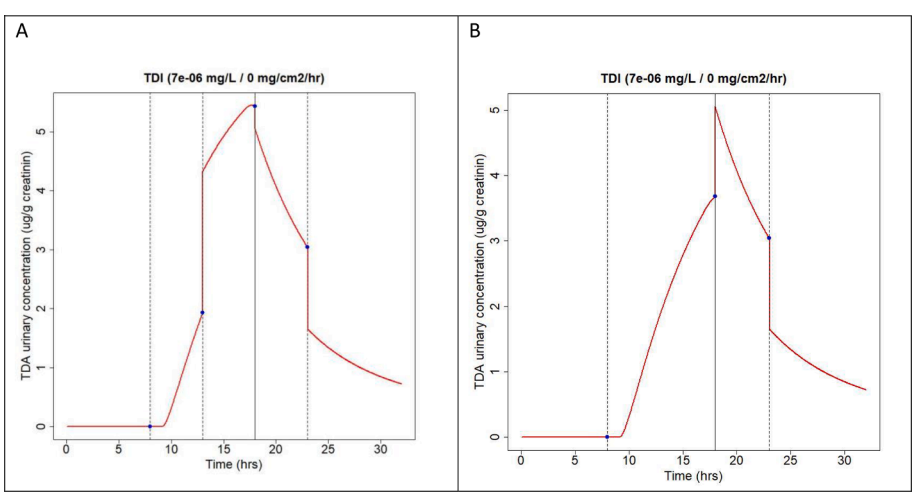

Fig. 7. Effect of the frequency of urination on predicted urinary concentrations after 8 h of exposure to TDI (7 μg/m^3). For the frequent urination scenario (A) urination was at 8:00, 13:00, 18:00, and 23:00, with exposure from 9:00 to 17:00. For the infrequent urination scenario (B) there was no urination at 13:00. Urination moments are indicated by dotted lines with predicted urinary concentrations indicated by a blue dot. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

The impact of differences in the frequency and timing of urination is illustrated in Fig. 7. In these scenarios workers were exposed to 7 μg TDI/m^3 for 8 h, but urination was either at 8:00, 13:00, 18:00, and 23:00 h ("frequent"), or only at 8:00, 18:00, and 23:00 ("infrequent"). Estimated TDA concentrations in urine obtained at the last urination moment were lower in the "infrequent" urination scenario (3.7 μg/gr creatinine; Fig. 7b) than in the "frequent" urination scenario (5.4 μg/gr creatinine, Fig. 7a).

Figure S4 in the appendix (Appendix H) shows results for the same exposure scenarios for MDI. Estimated MDA biomarker concentrations in post-shift samples were considerably higher for the "early" than the "late" scenario and also much higher for the "frequent" than the "infrequent" urination scenario illustrating that the effect of different exposure and urination scenarios on biomarker concentrations in post-shift samples critically depends on the kinetics of the isocyanates involved.

4. Discussion

4.1. Main findings

In this study we developed a PBK model for MDI and TDI; more specifically we modelled the renal excretion of MDA and TDA as degradation products. Global sensitivity analysis was used to assess which parameters contributed most to the uncertainty in simulated biomarker levels. Highly influential parameters included the fraction of diisocyanate absorbed, a subject's body weight, the binding rate of diisocyanates to albumin and other macromolecules, and (for MDI only) the transfer rate of macro-molecule bound MDI from the interstitial to the vascular lung compartments. We performed additional sensitivity analyses to investigate the importance of the timing of exposure and frequency and timing of urination and found that these had considerable impact on modelled urinary biomarker levels.

4.2. Model comparison

4.2.1. Inhalation

To be able to validate our model we compared model predictions with published data (Fig. 2A and 2B). This is not straightforward because PBK models are generally compared to experimental animal studies with a clearly defined exposure regime, whereas in this case, we aimed to use biomonitoring data to estimate exposure in chronically exposed human workers, which is more challenging for a chemical with both a fast and accumulating excretion pattern. The studies we extracted differed in terms of measuring exposure (task based versus TWA), the use of RPE (no use, very strict use, or worn inconsistently) and even in sample hydrolysis procedures. All these factors could impact urinary amine levels. For example, alkaline hydrolysis has been reported to give higher results as more adducts are released in comparison to acid hydrolysis (as reviewed in Scholten et al. 2020). This is also evident in Fig. 2A and 2B, where we found that of the three studies that used acid hydrolysis (i.e. Maître et al. 1993; Swierczyńska-Machura et al. 2015; Bello et al. 2019), two described lower urinary concentrations compared to model estimates. All other studies (that used alkaline hydrolysis), predicted higher urinary concentrations compared to model predictions.

The use of RPE could arguably result in lower urinary amine concentrations in comparison to our model predictions, unless the RPE was not very effective or not worn throughout the working period. And converting a task exposure to a TWA is only reliable if there was no exposure during the rest of the working period. We could not specify one specific parameter that was predictive of urinary measurements. For example, the Geens et al. (2012) study reported reasonably comparable urinary concentrations as we modelled (Fig. 2A) but the workers in that study did wear RPE (which could have resulted in lower levels), and reported a task based estimate (which we converted to 8 h (Sabbioni et al. 2017)exposure) and used the higher release alkaline method in comparison to the other TDI biomonitoring studies. Another difficulty is that levels of predicted urinary levels after longer exposures are difficult to compare because it is unclear whether exposure levels as measured on study days were representative for the entire period.

We further estimated accumulation due to binding to protein adducts by modelling urinary results on Monday morning after a weekend with no exposure. Accumulation was about 1.5 μg/gr creatinine for TDA (based on chronic exposure to 20 μg/m^3 TDI), and 0.18 μg/gr creatinine for MDA (based on chronic exposure to 1.25 μg/m^3) (Fig. 3A and 3B). A couple of studies measured pre-shift levels. For TDI, Monday morning urinary concentrations were reported to be 4.5 μg/gr creatinine (with Friday post-shift air measurements of 39.45 μg/m^3) (Geens et al. 2012), and between 0.15 and 4 μg/gr creatinine with air measurements between 0.52 and 4.1 μg/m^3 for 2,4-TDI (Tinnerberg et al., 2014). For MDI, Tinnerberg et al. (2014) reported 0.52 μg MDA/gr creatinine (and air sample measurements between 0.04 and 9.7 ug 4,4′-MDI/m^3). Based on these studies our model estimates seem to be in the same range as the published data, considering the various exposure scenarios.

There are several studies that measured diisocyanate-albumin specific adducts in plasma. We found two relevant studies, by (Sabbioni, Dongari, and Kumar 2010; Sabbioni et al. 2017). Both reported MDI-lysine concentrations in workers, ranging from 36.1 to 501 fmol/mg. This equals approximately 0.0005 to 0.0074 mg/L, assuming albumin concentrations in plasma of 40 g/L, and using the molecular weight of MDI-lysine. Our model estimates MDI albumin adducts level of approximately 0.0015 mg/L (after accumulation has stabilized) following repeated occupational exposure to 1.25 μg/m^3 MDI (Fig. 3D).

In general, for the urinary data, we can conclude that published results are reasonably comparable, although systematically lower, to our model predictions, but biomonitoring studies for diisocyanates could be more comparable if the studies were more harmonized and reported all relevant information, including the moment of exposure when it concerned a task based exposure measurement. Recently a protocol for measuring and reporting on diisocyanates was published, which is helpful in harmonizing data collecting and reporting (Jones et al. 2022).

4.2.2. Dermal

The dermal compartment of our model is difficult to evaluate. Henriks-Eckerman *et al.* (2014) reported average urinary concentrations of about 0.30 μg/gr creatinine for workers that did not use either gloves or RPE. We estimated levels of 0.02 and 0.410 μg/gr creatinine, directly after exposure and 4 h later, when considering the same inhalation and dermal exposure as reported by Henriks-Eckerman *et al.* (2014). There is evidence that most skin absorbed diisocyanate is held in a skin "reservoir", which could result in subsequent slower desorption into the circulation (ATSDR 1998). With our model we estimate a relative contribution of dermal exposure to total urinary excretion (within 72 h after exposure) of approximately 68%. Furthermore we predicted that, from the applied dose, 8.8% of the total dermal dose had been excreted within 72 h. This seems much higher in comparison to a controlled human exposure study by Hamada et al. (2018), who dermally exposed 4 volunteers for 8 h to MDI. They found very low amounts in urine and plasma of (about 0.01 to 0.2% of the applied dose). The authors hypothesize that MDI is rapidly absorbed but remain bound to the upper layers of the skin. Hoffmann et al. (2010) studied dermal absorption in rats and also found very low dermal absorption of MDI: at or below 1% of the applied dose. It has been argued that dermal exposure could lead to exposure via hand-to-mouth behavior. Currently our model assumes that there is no uptake in the gut after oral exposure, i.e. no systemic availability. To our knowledge there is no data available on absorption from the gut for MDI. For TDI, a study in orally exposed male rates found that 81% of the administered 14C- 2,4-TDI was recovered in feces, and only 8% in urine (Gledhill et al. 2005). Note that we did not consider hydrolysis of amines in vivo: for TDI this is supported by studies that describe that the majority of TDI was conjugated to macromolecules in urine after inhalation exposure (Health Council of the Netherlands 2018;

ATSDR 1998). Also for MDI the evidence of hydrolysis in vivo is uncertain: although Schütze et al. (1995) and Sepai, Henschler, and Sabbioni (1995) found free MDA and AcMDA in blood and urine after MDI exposure, a later study did not confirm these findings (Sennbro et al. 2003). We do provide an example approach for including hydrolysis using a liver compartment in Appendix A (liver metabolism).

4.2.3. Strengths and weaknesses of model, and possibilities

The strengths of our model include the potential to model both inhalation and dermal exposure, and to account for longer exposure histories. The biologically interpretable parameters can be validated in mechanistical studies. Weaknesses of our model include the fact that for many parameters there is uncertainty regarding the exact value, as also reflected in the wide parameter distribution range (e.g. confidence interval in Fig. 2A, 2B). In addition model validation was difficult because often information on the precise exposure situation was missing, for example on the moment of exposure when it concerned a TWA, or to which extent PPE was used throughout the day. For model calibration it would be helpful to have an individual dataset available with both external exposure estimates (quantitative air and dermal measures) including data on the exposure scenario (i.e. moment and duration of exposure) and individual behavior and information (i.e. moment of urination, body weight), together with well described biomonitoring data. This information could then be included in the model and as such reduce uncertainty of our common assumptions on e.g. body weight distribution or moment of urination. In addition if urinary values are measured over an extended period of time, even during/after a period of non-exposure, this provides information on the accumulation and binding to albumin, but also on the fraction absorbed. With a fully validated and calibrated model we have more certainty with regards to extrapolating across dose levels and exposure scenarios, and characterizing potential contributions of RPE and/or dermal exposure. In addition we could apply exposure reconstruction with more precision, when only urinary data is available. Lastly our model could be used for informing biological limit values (BLV). The BLV for both TDI and MDI are based on correlation formulas between air sampling and urinary data (Hartwig and MAK Commission 2021; DFG 2007). These correlations formulas were used to estimate urinary amine levels upon exposure to available MAKs. We compared the current available BLVs for diisocyanates to our model predictions. The Deutsche Forschungsgemeinschaft (DFG) derived a value of 6.13 μg TDA/gr creatinine based on the MAK value of 7 μg/m^3 and proposed a BLV of 5 μg TDA/gr creatinine (Hartwig and MAK Commission 2021). With our model we predicted urinary values of 6.90 μg TDA/gr creatinine, when modelling the last post-shift urine sample, so comparable tothe value derived using the correlation formula. The DFG also used correlation formulae for MDI in air and MDA in urine and proposed a value of 10 μg MDA/gr creatinine based on exposure to MDI at the MAK value of 0.05 mg/m^3. With our model we predicted MDA urinary values of 20.9 μg/gr creatinine, post-shift.

Recently the European Risk Assessment Committee (RAC) concluded that occupational diisocyanate exposure greater than 0.67 μg/m^3 NCO during an entire working life was likely to result in a more than 5% excess risk of developing bronchial hyperresponsiveness (BHR) (RAC 2020b). For TDI and MDI our model predicts urinary levels of 1.58 and 0.89 μg/gr creatinine for TDI and MDI respectively, at this critical exposure level. A purely health based BLV could then accordingly also be lower than the current values of 5 μg TDA/gr creatinine and 10 μg MDA/gr creatinine.

Declarations of interest.

None.

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Disclaimer

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Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envint.2023.107917.

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