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IMPROVED SCIENTIFIC BASIS FOR HUMAN HEALTH RISK ASSESSMENT FACTORS BY TOXICOKINETIC POPULATION MODELING

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To my family

POPULÄRVETENSKAPLIG SAMMANFATTNING

Dagens samhälle är till stor del beroende av kemiska ämnen. De finns i låga halter i praktiskt taget allting som används i det vardagliga livet; till exempel i mat, vatten, hygienprodukter, kläder och leksaker. Många exponeras också för höga halter av kemikalier i sin arbetsmiljö. Vissa av kemikalierna kan vara skadliga för hälsan. Hur skadliga de är beror bland annat på ämnets inneboende egenskaper och hur hög exponeringen är, samt hur ofta och länge som man exponeras (dosen). Hur mycket av ämnet som tas upp av kroppen och som når det organ där det utövar sin effekt (måldosen) styr också hur skadligt ämnet är. Individuella skillnader i till exempel ålder, kön, arvsanlag och hur fysiskt aktiv man är påverkar måldosens storlek och därmed också hur allvarliga hälsoeffekterna blir.

För att skydda människor från hälsoproblem som orsakas av kemiska ämnen sätter myndigheter gränsvärden som reglerar hur hög exponeringen får vara, till exempel i arbetslivet. Kunskapen om hur och vid vilken exponeringsnivå människor påverkas av olika kemiska ämnen är emellertid begränsad. Gränsvärden tas därför oftast fram genom att dela den högsta dos som inte har någon effekt i försöksdjur med ett antal s.k. bedömningsfaktorer. I de flesta fall används schablonvärden för att ta hänsyn till skillnader mellan djur och människor och skillnader mellan individer. Sedan dessa togs fram på 1950-talet har mängden information om kemiska ämnen, deras påverkan på hälsan och skillnader i känslighet mellan människor ökat markant.

Syftet med de studier som ingår i avhandlingen var att utveckla en metod för att ta fram vetenskapligt baserade bedömningsfaktorer som beskriver skillnader i måldosen mellan människor. Metoden tillämpades sedan på fyra vanliga organiska lösningsmedel; aceton, toluen, styren och metylklorid.

I Studie I utvecklades en matematisk modell som räknar ut måldosen efter exponering för aceton. Modellen baserades på kunskap om människans anatomi, om olika biologiska och kemiska processer samt på acetons specifika egenskaper. För att kunna använda matematiska modeller i arbetet med att sätta gränsvärden är det viktigt att först verifiera dem med hjälp av experimentella observationer. I Studie II användes därför ett statistiskt ramverk för att jämföra och uppdatera de för aceton beräknade måldoserna med mätdata från friska frivilliga försökspersoner som exponerats för aceton. Den uppdaterade modellen användes därefter, tillsammans med andra, redan utvecklade modeller för toluen, styren och metylklorid, för att simulera måldosen hos olika befolkningsgrupper (Studie III och IV). Vi undersökte bland annat vilken effekt ålder och kön hade i den allmänna befolkningen och vilken effekt kön, variationer i exponeringsnivå och i fysisk arbetsbelastning över tid hade på måldosen hos yrkesexponerade.

Resultaten tyder på att schablonvärdena är tillräckliga eller något höga för allmänbefolkningen, även då man tar hänsyn till skillnader i ålder och kön. För

yrkesmässigt exponerade kan de däremot vara otillräckliga då exponeringsnivån varierar över tid, speciellt om den fysiska arbetsbelastningen också varierar.

Studierna som ingår i den här avhandlingen kan bidra till att öka tillförlitligheten hos de gränsvärden som reglerar exponering för kemiska ämnen. Genom att ersätta schablonvärdena med vetenskapligt baserade bedömningsfaktorer kan för höga gränsvärden, med försämrad hälsa hos befolkningen och stora kostnader för samhället, förhindras. Onödigt låga gränsvärden, med långtgående ekonomiska konsekvenser för både näringsliv och samhälle, kan också undvikas.

ABSTRACT

Exposure limits or guidelines are derived to protect humans from adverse effects caused by exposure to chemical substances in the environment or at the workplace. The internal dose of a chemical is determined by toxicokinetic (TK) processes such as uptake, distribution and elimination, and is closely related to the risk of adversity. The internal dose varies among individuals due to differences in age, genetics, physical activity, health status and life-style. Thus, it is important to address population variability for the exposure limits to be protective. TK variability is typically accounted for by the use of a default assessment factor of 3.16. However, the scientific basis of the exposure limits may be improved by replacing the default value with a chemical specific adjustment factor ($CSAF_{HK}$), derived from experimental data. By doing so, more appropriate exposure limits are achieved, and large costs for society associated with both too high and too low exposure limits may be avoided.

Substitution of the default value is often obstructed by the lack of suitable experimental data. In this thesis, this limitation was addressed by the development of a probabilistic framework using physiologically based pharmacokinetic (PBPK) modeling. It was used to derive $CSAF_{HK}$ for four commonly used organic solvents; acetone, toluene, styrene and methyl chloride.

PBPK models based on information on anatomical, physiological and biochemical parameters were used to calculate the internal doses following inhalation exposure to the four chemicals. A description of washin-washout in the respiratory tract was evolved for polar solvents such as acetone. Additional information on the model parameters contained in human experimental toxicokinetic data was taken advantage of by Bayesian analysis. Meanwhile, the methodology was explored with respect to prior assumptions. $CSAF_{HK}$ were derived from population distributions of internal dose obtained by Monte Carlo (MC) simulation from distributions of the model parameters. The influence of age and gender on the internal dose was slight. Thus, the factors obtained for all substances were below 2.5. However, the effects of fluctuations in exposure level and workload increased the $CSAF_{HK}$ up to 6.1, indicating that workplace exposure may need specific attention. Given the diverse properties of acetone, toluene, styrene and methyl chloride, the results can probably be generalized to most organic solvents and similar chemicals.

The $CSAF_{HK}$ presented in this thesis are derived from extensive information on intraspecies toxicokinetic differences and cover the effects of common toxicokinetic modifiers. Thus, they are well suited to replace the default value. The population framework may be further extended to include other chemicals, as well as additional experimental data on population variability when such becomes available.

LIST OF PUBLICATIONS

This thesis is based on the following publications;

I. **Mörk A-K**, Johanson G.
A human physiological model describing acetone kinetics in blood and breath during various levels of physical exercise.
Toxicol Lett. 2006 Jun 20;164 (1):6-15.

II. **Mörk A-K**, Jonsson F, Johanson G.
Bayesian population analysis of a washin-washout physiologically based pharmacokinetic model for acetone.
Toxicol Appl Pharmacol. 2009 Nov 1;240(3):423-32.

III. **Mörk A-K**, Johansson G.
Chemical-specific adjustment factors for intraspecies variability of acetone toxicokinetics using a probabilistic approach.
Toxicol Sci. 2010 Jul;116 (1):336-48.

IV. **Mörk A-K**, Jonsson F, Johanson G.
Chemical-specific adjustment factors for toluene, styrene and methyl chloride by population modeling of toxicokinetic variability.
Manuscript.

The publications are referred to by their roman numbers (I-IV) in the thesis text. The articles are reproduced in full text as appendices.

CONTENTS

LIST OF ABBREVIATIONS

ACGIH	American Conference of Governmental Industrial Hygienists
AF	Assessment factor
AUC_{24h}	The 24 hour integrated concentration of parent compound in blood
BMI	Body Mass Index
BOA	Bayesian Output Analysis Program
BH	Body height
BW	Body weight
C_{Max}	Maximal concentration of parent compound in blood
CNS	Central nervous system
CSAF	Chemical specific adjustment factor
$CSAF_{HK}$	Chemical specific adjustment factor for human toxicokinetic variability
CYP450	Cytochrome P450 superfamily
Dutch TNO	Netherlands Organization for Applied Scientific Research
ECETOC	European Center for Ecotoxicology and Toxicology of Chemicals
ECHA	European Chemicals Agency
GST	Glutathione transferase
IPCS	International Programme on Chemical Safety
LOAEL	Lowest observed adverse effect level
MC	Monte Carlo (simulation)
MCMC	Markov chain Monte Carlo (simulation)
Met_{24h}	Amount metabolized during 24 hours, normalized to $BW^{0.75}$
NIOSH	National Institute of Occupational Safety and Health (United States)
NOAEL	No observed adverse effect level
NOES	National Occupational Exposure Survey
P_{BA}	Blood: air partition coefficient
PBPK	Physiologically based pharmacokinetic model
PBTK	Physiologically based toxicokinetic model
P_{FatB}	Fat: blood partition coefficient
PVR	Perfusion over ventilation ratio
REACH	The EU regulation “Registration, Evaluation, Authorization and Restriction of Chemicals”
RfC	Reference Concentration (set by the US EPA)
TLV	Threshold Limit Value (set by the ACGIH)
US EPA	United States Environmental Protection Agency

1 INTRODUCTION

Chemical substances are essential parts of human lives and abundant in our surroundings. Thus, humans are exposed to them on a daily basis in occupational as well as in residential settings. Chemicals can be harmful to human health, depending on their inherent properties, the exposure scenario, and on individual susceptibility and behavior.

Health risk assessment is used to determine if a particular chemical is harmful to human health and, if so, under what circumstances. This information is used to derive exposure standards aiming at protecting most individuals in a population from the harm of exposure. In such efforts, information on human variability in response to chemical exposure is important. Human exposure standards are typically based on the highest dose or exposure level at which the negative health effect is not detected in animals (No Observed Adverse Effect Level - NOAEL). To determine an exposure level which is considered to be safe for humans, the NOEAL is divided by a number of assessment factors.

A so-called default assessment factor (AF) of 10 has traditionally been used to address human variability. The default AF is based on limited scientific information, thus making its use in human health risk assessment associated with considerable uncertainty. In recent years, possibilities to replace the default value with more realistic, data-driven ones have emerged by the increase of available experimental toxicological data to quantify human variability, and advances in modeling and computer simulation.

Improving the scientific basis for assessment factors would contribute to more reliable exposure standards for chemical substances. Thus, too high exposure standards resulting in illness in a population could be avoided. Illness is not only affecting the wellbeing of individuals, but also has high costs for society. Unnecessarily low exposure standards could also be avoided. Such standards may have far reaching economic consequences for industry, as well as for society.

2 AIM

The overall aim of this thesis was to improve the scientific basis of human health risk assessment factors by quantifying intraspecies (human to human) variability in the toxicokinetics of organic volatiles.

The specific aims were to;

- construct a physiologically based pharmacokinetic (PBPK) model for the inhalation of polar organic solvents, using acetone as an example (paper I)
- calibrate the PBPK model for acetone to human experimental toxicokinetic data using Bayesian analysis (paper II)
- develop a probabilistic framework for the derivation of chemical specific adjustment factors for human toxicokinetic variability ($CSAF_{HK}$) using acetone as an example (paper III)
- derive $CSAF_{HK}$ for non-polar solvents such as toluene, styrene and methyl chloride by deploying existing PBPK models and the probabilistic framework (paper IV)
- evaluate the effect of age and gender on intraspecies toxicokinetic variability (paper III and IV)
- investigate the effect of various workplace conditions, such as ventilation rates, fluctuations in exposure level and workload on inter-worker toxicokinetic variability (paper III and IV)

3 BACKGROUND

3.1 ORGANIC SOLVENTS

Organic solvents are liquids capable of dissolving other organic substances. Due to their attractive properties they are commonly used in industrial processes and in manufacturing of a wide range of products. They are also found in many household commodities. Since the use of organic solvents is so common, the exposure to humans is widespread.[1] For example, it has been estimated that 10 million US workers are potentially exposed to organic solvents.[2] In the United Kingdom 8 percent of the working population is thought to regularly handle organic solvents.[3] Organic solvents can cause both acute and chronic health effects. Acute health effects typically follows from a single significant exposure and are often reversible. Common acute effects from solvent exposure are for example irritation of the eye, nose, and throat as well as headaches, loss of coordination and nausea.[4,5] Conversely, chronic health effects are caused by prolonged or repeated exposures over many days, months or years. Thus, they typically do not occur immediately and are often irreversible. Chronic health effects from solvent exposure include among other damage to liver, kidney and central nervous system (CNS), and cancer.[5-9]

3.2 DOSE - RESPONSE

Exposure to organic solvents may be harmful to human health. The risk of adversity is determined by a number of factors, such as the inherent properties of the substance and the dose, i.e. the magnitude, duration and frequency of the exposure. Adversity is also dependent on the toxicokinetics of the substance, i.e. how much of the substance that enters the body and what happens to it well inside, as well as on how the chemical interacts with the body or its mode of action (toxicodynamics). Although the dose is the same, the magnitude of the response will typically vary among humans as a consequence of individual differences in the kinetic and/or dynamic processes. Variation in response may also be caused by differences in susceptibility and behavior.

3.3 HUMAN HEALTH RISK ASSESSMENT

Toxicological risk assessment may be performed to determine the probability of chemical substances to cause adverse health effects in a human population of concern. It involves determining the inherent potential of the chemical substance to cause harm, the human dose-response relationship and, the likelihood and the extent of the exposure in the population.[10]

Dose-response assessment typically starts by identifying a critical health effect and a dose or exposure level at which this effect is not detected (NOAEL) or found to be minimal (Lowest Observed Adverse Effect Level - LOAEL).[10] In this process, priority is generally given to human data. However, as such studies are rare studies of effects in experimental animals are in most cases used instead.

To convert the NOEAL or LOAEL value into an exposure level considered to be of no concern for humans, adjustments may be necessary. They are typically performed for the use of animal data, to cater for variability between humans and for other data lacks, such as uncertainties relating to exposure duration or route.[11] For this purpose, assessment (uncertainty, adjustment, extrapolation, safety, conversion) factors are used by considering the possibility that humans may be more sensitive than animals to the negative effects of chemical substances. Thus, to derive human exposure standards, the NOAEL/LOAEL is divided by the product of the assessment factors (AF);

$$\text{Human exposure standards} = \frac{\text{NOAEL}}{\prod \text{AF}} \quad (1)$$

3.3.1 Assessment factors

Default assessment factors

Default assessment factors are numerical values traditionally used to compensate for the lack of full information on the effects of chemical substances in human populations.[12-14] They were first introduced in the United States in the mid-1950s by Lehman and Fitzhugh.[15] The suggested 100-fold factor was derived based on a comparison of the knowledge of the toxicity of fluorine in rats and arsenic in dogs with the supposed toxicity in humans. Although based on a comparison between species, the factor was also considered to compensate for variability within a population.[11,14,15] Since then, the default factor of 100 has been slightly modified to separately account for interspecies (animal to human) and intraspecies (human to human) variability. Values of 10 were suggested for both adjustments.[16,17] The factors are commonly assumed to be independent and are hence multiplied.

Chemical specific adjustment factors (CSAF)

Default AFs are deployed to compensate for lack of knowledge. Thus, when relevant information is available it should be used instead.[18] To enable data describing differences from toxicokinetic or toxicodynamic effects to be incorporated separately, the default uncertainty factors of 10 were further sub-divided.[19-21] For intraspecies variability, factors of 3.16 ($10^{0.5}$) were derived for toxicokinetic and toxicodynamic effects, respectively.[10,21,22] The subdivision of the 100 fold factor is depicted in Figure 1.

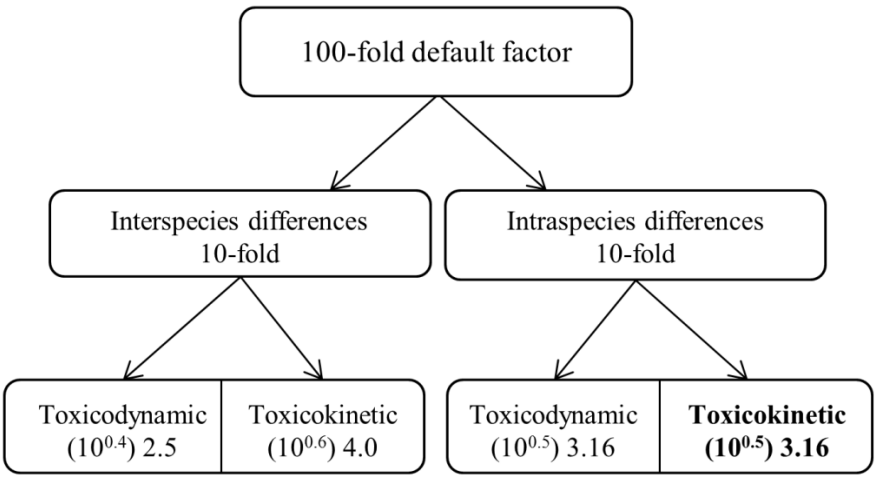

Figure 1. Division of the 100-fold factor according to WHO/IPCS.[21] This thesis focuses on the highlighted part.

General population and workers

Health risk assessments typically aim at the general or the occupationally exposed populations. The two populations differ in a number of ways, the most important being the exposure scenario and the potential susceptibility of the individuals.

Exposure to the general population is often continuous and life-long to low levels of chemicals whereas in an occupational setting the exposure is shorter termed and repeated (generally 8 hours a day, 5 days a week). Workers are typically exposed to higher levels than the general population and the exposure is more often carried out during strenuous physical activity. The general population is more heterogeneous than the occupationally exposed population as it includes individuals who might be especially sensitive to the exposure of chemical substances, such as children, the elderly and individuals with pre-existing medical conditions. Workers are typically healthy adults.

These differences are sometimes reflected in the size of the default value applied by different exposure standard setting organizations. Thus, the guidance document of the European REACH regulation proposes default AFs of 5 and 10,[23] the Dutch TNO (Netherlands organization for Applied Scientific Research) has proposed AFs of 3 and 10, and the European Centre for Ecotoxicology and Toxicology of Chemicals (ECETOC) has suggested AFs of 3 and 5, for the working and general population, respectively. An additional AF has sometimes also been applied to protect children and infants as they may be especially susceptible to the harm of chemical substances during development.[24,25]

Following the same principle as for the subdivision of the default assessment factor of 10 (section 3.3.1), the toxicokinetic moiety for inter-worker variability would be between 1.7 and 2.2 ($3^{0.5}$ and $5^{0.5}$).

3.4 TOXICOKINETICS OF ORGANIC SOLVENTS

Exposure standards or guidelines have traditionally been based on measures of external dose, for example the administered dose or the concentration of a chemical substance in ambient air. However, adversity is often more closely related to the amount of the chemical substance that reaches the tissue or the organ where it exerts its effect (internal dose). The internal dose is determined by biological and physiological processes which govern the uptake, distribution and elimination of the chemical.

3.4.1 Uptake

This thesis focuses on uptake via inhalation, the most common route of exposure to organic solvents.[26] However, they are also taken up via the skin.[27] Organic solvents are typically adsorbed by passive diffusion along their concentration gradient. The amount which is taken up by the body depends on the characteristics of the solvent and the barriers it has to cross to enter.[28] During inhalation, air, together with any volatile it contains, travels via the nose and mouth to the trachea and further into the bronchi (Figure 2). The bronchi branches dichotomously in the lungs and become increasingly smaller in diameter until they end in small air sacs, called alveoli. The walls of the alveoli are very thin and richly supplied with capillaries. They cover up to 90 percent of the alveolar surface and receive the entire cardiac output.[29,30] Thus, chemical vapors can easily travel through the walls of the alveoli to enter the systemic circulation.

The respiratory airways are lined with a thin layer of epithelial tissue which secretes mucus. The function of the mucosa is to condition the inhaled air with moisture and to trap pathogens, dirt, and particles.[31] Polar (hydrophilic) solvents may be absorbed by the mucosa during inhalation, and subsequently be released during exhalation (washin-washout effect). Hence, a fraction of the inhaled solvent vapor will not participate in the gas-exchange in the alveoli.[32,33] However, it may still reach the systemic circulation via exchange trough the bronchial walls.[34-37]

3.4.2 Distribution

Once the solvent has reached the systemic blood flow it is carried throughout the body and into organs and tissues. The extent of the distribution is determined by the solvent's properties and the characteristics of the tissue membranes.[28] Distribution of organic solvents typically occurs by passive diffusion.

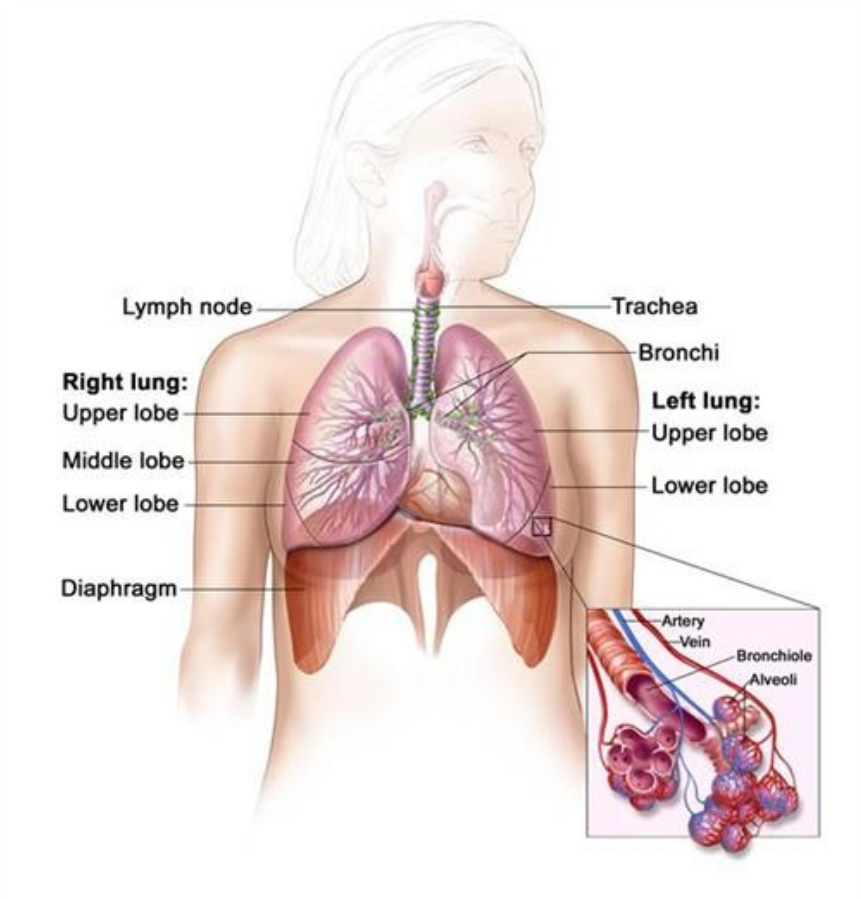

Figure 2. The respiratory airways. Image from Wikimedia.[38]

3.4.3 Elimination

Elimination of chemical substances occurs via metabolism and excretion. During metabolism, solvents are transformed into more hydrophilic compounds. Such compounds are generally less toxic and easier for the body to dispose of. However, some chemicals undergo metabolic activation. In such cases the metabolite is more toxic than the parent compound and thus responsible for the toxic effect. There are typically two phases in the biotransformation process. The function of phase I reactions is to add or expose functional groups to which hydrophilic molecules can be bound by phase II reactions.[28] The phase I, cytochrome P450 (CYP450) reactions are thought to be the most important in the metabolism of organic solvents.[39] The biotransformation enzymes are mainly present in liver, but large amounts also exists in lung, kidney and in the skin. Small amounts can be found in almost any tissue in the human body.[26] Organic solvents are excreted as such, or as a metabolite after biotransformation. Excretion occurs into urine via the kidneys or into feces via the biliary route. Unchanged solvent may also be exhaled.

3.4.4 Intraspecies variability

The extent of the uptake, distribution and elimination of chemical substances varies between individuals. As a consequence, the magnitude of the internal dose and hence the risk of adversity will vary within a human population. Factors that have been shown

to modify the kinetics of inhaled organic solvents in humans include among others; age, gender, physical exercise, and metabolism.[26,40-43] Variability may also be due to lack of knowledge, i.e. uncertainty.[44,45]

Age

Substantial differences in the toxicokinetics of solvents may occur as a consequence of age.[43] Due to their rapid development, children are also more variable within a defined age group than adults.[46] Children may have a larger pulmonary uptake than adults as they breathe more relative to their body size.[47] Children also tend to be more physically active than adults.[48] During physical activity, lung ventilation increases. This may increase the uptake of inhaled volatiles in children as compared to adults. Children also have different proportions of fat, muscle and water as compared to adults. The body water content in children is higher, and it also varies greatly with age.[49,50] Fat content also varies during development.[50] Such differences may affect the distribution of organic solvents. In general, children metabolize more relative to their body size. The increased metabolism has been argued to compensate for any increased exposure.[51] However, in cases where the metabolite is responsible for the toxic effect, the increased metabolic capacity may put children at larger risk of adverse health effects. Moreover, metabolism and excretion in very young children may be immature.[52-54] For example, the levels of the CYP450 enzymes reach adult levels first at the age of 2-6 months.[52] The activity of other important biotransformation enzymes may also be decreased in young children.[43]

Gender

Most of the observed gender differences of the toxicokinetics of organic solvents may be explained by differences in body size, however not all of them. In general, females have a higher percentage of body fat than men. As a consequence, they may receive higher internal doses of lipophilic solvents. Moreover, females may have a prolonged internal exposure to lipophilic substances after the end of the ambient exposure due to the increased storage opportunity.[26]

Physical activity

Exposure to organic solvents typically occurs during the performance of various tasks. These tasks may require various levels of physical activity. Physical activity has been shown to alter the toxicokinetics of a large number of volatiles.[26,37,40,55] An increase in workload increases pulmonary ventilation and cardiac output.[37] The cardiac output is also redistributed, with active muscles, fat and skin receiving relatively larger portions of the blood flow whereas the blood flow to liver, gastro-intestinal tract and kidneys decreases.[37,40] The metabolism can thus be affected indirectly by the decrease in blood flow to liver. Elimination of solvents by exhalation may be increased by an increase in ventilation.[26] The influence of physical activity on the toxicokinetics is chemical dependent, however in general, exposure during physical exercise results in an increase of the internal dose and higher risk of adverse effects compared to exposure at rest.[26,40]

Metabolic capacity

Different members of the cytochrome P450 enzyme family (CYP) are primarily responsible for the biotransformation of organic solvents in humans. In addition, several other enzymes are involved. Thus, intraspecies differences in the toxicokinetics of organic solvents may partly be explained by differences in metabolic activity. For example, up to 10-fold differences in CYP activity has been seen in humans.[56,57] Genetic polymorphisms have been detected for several enzymes involved in the biotransformation of organic solvents, such as CYP1A1 and GSTT1.[58] Individuals can either be poor, fast or non-metabolizers dependent on the number of copies of the functional gene. More than a tenfold variation in metabolic capacity due to genetic polymorphism in biotransformation enzymes has been reported.[59] At low exposure levels, a linear relationship between the exposure level and internal dose can be expected. However, at higher exposure levels metabolism can become saturated. Saturation results in a disproportional change of the internal doses metrics and may contribute to a higher intraspecies variability in toxicokinetics of organic solvents.[26]

Measurement uncertainty

The experimentally determined concentrations of a chemical substance in a biomarker (blood, breath or urine) vary between individuals at the same exposure scenario. Likewise, the measurements of physiological parameters, such as ventilation rates or body weight vary among humans. Such differences may be due to biological variability, but could also be a consequence of erroneous or imprecise measurements, or most likely, the combination of both.[60] Thus, observed intraspecies differences can be attributed both to biological variability and uncertainty. Uncertainty can be distinguished from biological variability as it can be reduced by performing new experiments or by gathering more information. Biological variability on the other hand is an intrinsic property of living systems which size cannot be affected or altered by an increase in knowledge.

3.5 PHYSIOLOGICALLY BASED PHARMACOKINETIC MODELS

Physiologically based pharmacokinetic (PBPK) models attempt to describe the processes of uptake, distribution and elimination of a substance in mathematical terms, based on knowledge on human anatomy and physiological mechanisms. Thus, they can be used to calculate the internal dose of a chemical substance based on the external exposure. In the scientific literature, the term physiologically based toxicokinetic (PBTK) model is also used.

A typical PBPK model for inhalation exposure to non-polar solvent vapors is shown in Figure 3. It contains special compartments for exchange of solvent between inhaled air and systemic blood, and for the storage of solvent in fat. The metabolism of the solvent is assumed to occur in liver by a single pathway.

A PBPK model arranges the organs or tissues in compartments and links them with perfusing blood. The structure of the model is partly determined by its intended use, and partly by the knowledge of the kinetics of the substance of interest. Thus, the

model typically includes separate compartments for target tissues, tissues responsible for biotransformation, storage tissues and tissues for which experimental data is available. The model also includes the exposure route(s). Remaining tissues can be lumped in joint compartments based on similarities in blood flow rates and tissue; blood partition coefficients. Common practice is to minimize the complexity of the model to reduce data needs and computational time. The compartments are typically assumed to be well-stirred and perfusion rate limited. A mass-balance differential equation is used to describe the movement of the substance in each compartment. The set of differential equations are solved by numerical integration to predict the amount of chemical substance in the tissue(s) of interest over time.

Measurements of the physiological, physiochemical and biochemical parameters included in the PBPK model equations may be derived from the scientific literature, vital statistics, or other sources and incorporated in the model. These are for example the ventilation rate, organ volumes, perfusion rates, and the partition coefficients and metabolic parameters of the chemical substance.

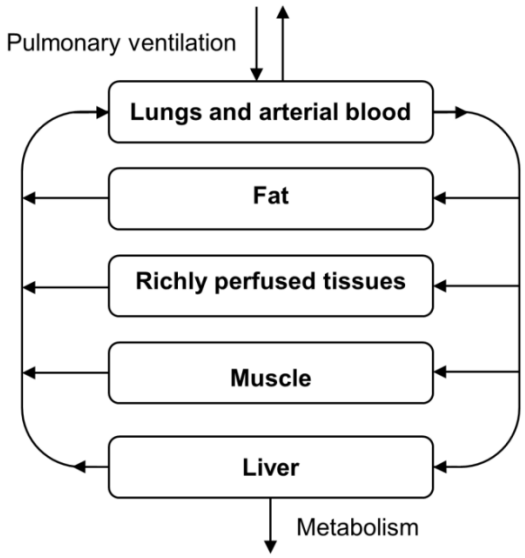

Figure 3. The structure of a typical PBPK model for an inhaled non-polar solvent.

To evaluate the performance of the PBPK model, it is often validated against experimental toxicokinetic data. In most cases it is necessary to refine the model due to disagreement between the model predictions and observations. Refinement can be achieved by changes in the model structure, and/or by calibration. During calibration, values or distributions of the model parameters that yield the best agreement between the model predictions and experimental observations are derived.

PBPK models are attractive tools in toxicological risk assessment.[61-64] Apart from being able to calculate the internal dose after a chemical exposure they are also applicable in many of the extrapolations performed in order to estimate human health risks from

experimental data. Since PBPK models are anatomically based and therefore the same for all mammals, they can be used to extrapolate between different species, age groups, gender and conditions by only changing the values of the model parameters.

3.6 MODELING OF TOXICOKINETIC VARIABILITY

Population variability of the internal dose can be estimated by Monte Carlo (MC) simulation from distributions of the PBPK model parameters.[60] The distributions describe the variability of the parameters in the human population. During MC sampling random values are iteratively drawn from the distributions and used as inputs to the PBPK model. Thus, the result obtained from such a simulation is a population distribution of internal dose.

To make accurate predictions of population dose metrics for regulatory use, it is essential to first calibrate the PBPK model to experimental toxicokinetic data.[65] Calibration of PBPK models is not an easy task. PBPK models often involve non-linear biological processes, co-varying model parameters, and different levels of biological variability in both model parameter distributions and experimental data. In addition, uncertainty due to lack of knowledge may be important. Calibration of PBPK models is therefore conveniently performed in a Bayesian hierarchical framework.[66]

Hierarchical (population) models

Hierarchical models describe the variability within a large population based on observations from few individuals.[67] The main idea is that a common model describes all individuals, but that the model parameters may differ between them. Some of the parameters are measured in the experimental studies and are considered known. By letting remaining, unknown, parameters belong to a statistical distribution, they can be estimated from the experimental data for the individual as well as for the population.[68] Meanwhile, different sources of variability, such as biological variability and uncertainty, can be separated.[44,69-71]

Bayesian inference

In a Bayesian analysis, the calibration of the hierarchical PBPK model to experimental toxicokinetic data is facilitated by the use of previous knowledge. Knowledge on the model parameters can be derived from the scientific literature. The previous knowledge is expressed by its probability and hence summarized as distributions. These prior parameter distributions are then updated with information in experimental toxicokinetic data to yield posterior distributions which are in agreement with both previous knowledge and the observations. The updating of the prior distributions is performed according to strict rules, given the likelihood of a given set of parameters to yield the observed experimental data.

Markov chain Monte Carlo simulation

Posterior distributions can be generated using numerical methods such as Markov chain Monte Carlo simulation (MCMC). In a first step, random parameter values are sampled from the prior distributions using Monte Carlo simulation to initiate one or

several Markov chains. The PBPK model makes a prediction of the time course of the chemical in blood based on the given parameter set. MCMC is an iterative process, thus for each iteration, new parameter sets are generated and new predictions are made. Based on how well the predictions and experimental observations agree, the new parameter set will be kept or rejected. Following a large number of iterations, the prior distributions will converge to create stationary distributions, called posterior distributions.[72,73] Convergence is assessed by one or several formal criteria.[74]

Following convergence, the chains can be run further to obtain population distributions of the model parameters. From these, population distributions of internal dose can be simulated by using the PBPK model and simple forward Monte Carlo sampling.

4 METHODS

4.1 MODELING SUBSTANCES

Acetone, toluene, styrene and methyl chloride are organic solvents commonly used in industry. Except for methyl chloride, they are also common in many household commodities. They are all high volume chemicals, with production exceeding 450 tons per year.[75] Besides having many similar properties, acetone, toluene, styrene and methyl chloride are also quite diverse. Thus, they allow for range of properties which may affect the toxicokinetics of organic solvents to be explored. The admissible exposure levels of the four chemicals vary between standard setting organizations and countries. In this thesis, the Reference Concentrations (RfC) for continuous exposure during a lifetime set by the United States Environmental Protection Agency (US EPA) were used to simulate exposure to the general population, whereas the threshold limit values (TLV) recommended by the American Conference of Governmental Industrial Hygienists (ACGIH) for average concentrations in air on the basis of a 8 h a day and 40 h per week work schedule were used for occupational exposure.

4.1.1 Acetone

Acetone is produced naturally by many plants and mammals, including humans, as a result of the breakdown and utilization of stored fats and lipids as a source of energy.[76] However, industrial processes contribute more acetone to the environment than natural processes. Acetone is used to produce for example plastics, fibers, drugs, and other chemicals. The toxicity of acetone is mediated by the parent compound and exposure to moderate-to-high amounts may cause effects on the CNS, and irritation of the respiratory tract and eyes.[77] Acetone is a polar solvent. Thus, it has high solubility in blood (blood; air partition coefficient P_{BA} of about 220) and poor solubility in fat (fat; blood partition coefficient, P_{FatB} of 0.23) (Paper II, Table 3). Acetone is metabolized by CYP2E1. Due to the lack of data, an RfC has not yet been established by the US EPA. Thus, in this thesis the suggested RfC of 29 ppm was used.[78] ACGIH recommends a TLV of 500 ppm for occupational exposure.[79] The National Institute of Occupational Safety and Health Administration (NIOSH) estimates that 1.74 million people, of which 31 percent are females, are occupationally exposed to acetone in the US.[2]

4.1.2 Toluene

Workers are mainly exposed to toluene is in the production of gasoline, but toluene is also frequently used to produce paints and lacquers.[80] Exposure to the general population is generally through the use of household products containing toluene, but cigarette smoke is also an important source. Both chronic and acute effects from toluene exposure are thought to be derived from the direct action of the parent compound on receptors in the brain.[81-83] Toluene is a non-polar solvent with low blood solubility, and high affinity for fat (P_{BA} of 16 and P_{FatB} of 54, Paper IV Table 4). Toluene is primarily metabolized by different members of the CYP family.[84] The RfC set by the US EPA is 1.3 ppm.[85] About 2 million US workers are estimated to be

exposed to toluene at their workplace.[2] 19 percent of these are females. The TLV recommended by ACGIH is 20 ppm.[79]

4.1.3 Styrene

Occupational exposure to styrene occurs through manufacturing and processing of polystyrene plastics and resins. The general population is exposed to styrene from anthropogenic origin and by cigarette smoke. The CNS depressant effect of acute exposures to styrene levels is probably due to direct effect of the unchanged styrene on nerve cell membranes. The chronic toxicity is however thought to be mediated through one or several metabolites.[86,87] Metabolism of styrene occurs mainly by members of the CYP family to styrene-oxide. The exact mechanism for the chronic effects of styrene exposure is not known, however binding of styrene-oxide to various components of the nerve tissue has been proposed as a probable mechanism.[88,89] Styrene is a non-polar solvent. It is moderately soluble in blood and has high fat solubility (P_{BA} of 64 and P_{FatB} of 49, Paper IV Table 4). The RfC is 0.24 ppm.[90] About 330 000 US workers are supposed to be exposed to styrene vapor at their work place. 26 percent of them are estimated to be female.[2] The TLV recommended for styrene is 20 ppm.[79]

4.1.4 Methyl chloride

The general population is primarily exposed to methyl chloride produced naturally by algae, kelp and fungi. However for smokers, cigarettes are probably the most important source. Occupational exposure to methyl chloride occurs mainly in the production of silicones.[91] Acute exposure to methyl chloride has caused severe neurological effects in humans. Methyl chloride is also known to cause effects on the heart rate, blood pressure, liver, and kidneys in humans. The toxic entity of methyl chloride is presumably a metabolite, however the mechanism of action is unknown.[92] Mehyl chloride is a non-polar solvent. It has low blood- and moderate fat solubility (P_{BA} of 1.7 and P_{FatB} of 6, Paper IV Table 4). Most of the inhaled methyl chloride is metabolized by GSTT1.[93] GSTT1 is polymorphic and have a tri-modal distribution reflecting fast, slow and non-conjugators.[94,95] The RfC is 0.04 ppm.[96] 10 000 workers in the US are potentially exposed to methyl chloride, of which 6 percent are female.[2] The recommended TLV is 50 ppm.[79]

4.2 HUMAN EXPERIMENTAL DATA (PAPERS I AND II)

The human toxicokinetic data for acetone came from a total of 18 male volunteers.[97,98] The volunteers were exposed while performing physical exercise on a bicycle ergometer. Extensive sampling of blood and breath was performed during and after the end of the exposure. The collection of exhaled air from a volunteer is depicted in Figure 4.

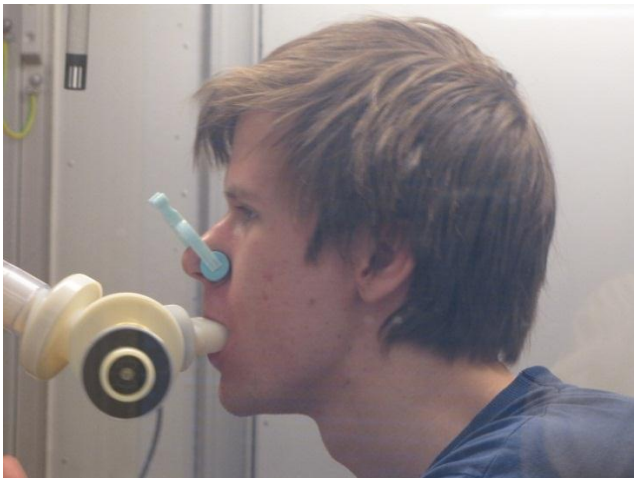

Figure 4. Sampling of exhaled breath through a mouthpiece.

In 1981, 8 male volunteers were exposed to acetone vapor at two separate occasions.[98] The first time all 8 volunteers were exposed at rest to approximately 550 ppm of acetone vapor (series I). On the second occasion, the exposure level was approximately 290 ppm during 2 hours. 4 of the volunteers were exposed at rest for 30 minutes followed by 50W workload for 90 minutes, and the remaining 4 volunteers were exposed while performing a stepwise increase in workload from rest to 50, 100 and 150 W. Each workload scenario lasted 30 min. In 1999, 10 male volunteers were exposed to approximately 250 ppm acetone for 2 hours during light physical exercise (50 W).[97]

4.3 PBPK MODELS

4.3.1 Acetone (Papers I, II and III)

The acetone PBPK model is based on a typical model for inhalation with compartments for lungs, richly perfused tissues, fat, liver, and muscles and skin (Figure 5). It accounts for the effect of washin-washout in the respiratory airways, physical exercise and endogenous acetone production. Washin-washout of solvent vapor in the lung is described by modeling the lung by using four separate compartments; bronchioles, mucosa, alveolae and arterial blood. Solvent vapor is allowed to be exchanged with the systemic blood in the bronchioles via the mucosa, as well as in the alveolar region.

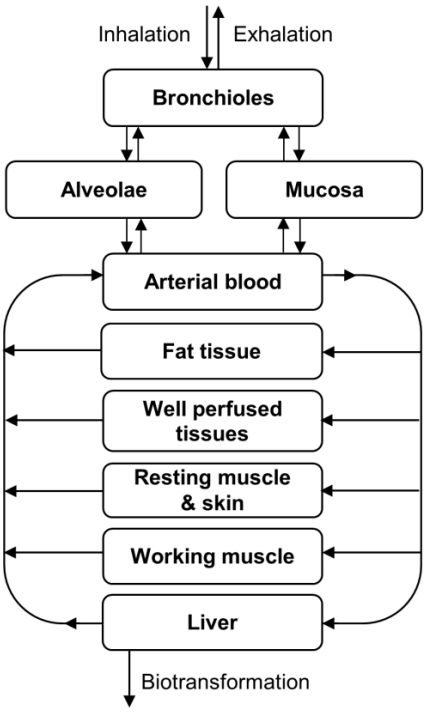

Figure 5. The acetone PBPK model structure. The washin-washout effect of solvent vapor in the upper respiratory tract is described by using four separate compartments for the lung.

The change in the concentration of acetone in the respiratory tract over time (*dC/dt*) is described by the following four differential equations:

$$\frac{d(C_{bro})}{dt} \cdot V_{bro} = Q_{alv} \cdot (C_{inh} + C_{alv} - 2 \cdot C_{bro}) - Q_{alv} \cdot (C_{bro} - \frac{C_{muc}}{P_{w:a}}) \quad (2)$$

$$\frac{d(C_{muc})}{dt} \cdot V_{muc} = Q_{alv} \cdot (C_{bro} - \frac{C_{muc}}{P_{w:a}}) + Q_c \cdot (\frac{C_{art} \cdot P_{w:a}}{P_{b:a}} - C_{muc}) \quad (3)$$

$$\frac{d(C_{alv})}{dt} \cdot V_{alv} = Q_{alv} \cdot (C_{bro} + \frac{C_{art}}{P_{b:a}} - 2 \cdot C_{alv}) \quad (4)$$

$$\frac{d(C_{art})}{dt} \cdot V_{art} = Q_{alv} \cdot (C_{alv} - \frac{C_{art}}{P_{b:a}}) + Q_c \cdot (C_{muc} - \frac{C_{art} \cdot P_{w:a}}{P_{b:a}}) - Q_c \cdot C_{art} + \sum Q_t \cdot \frac{C_t \cdot P_{b:a}}{P_{t:a}} \quad (5)$$

where *Q* and *P* signifies flows and partition coefficients respectively. *C* and *V* denotes concentrations and volumes. Subscripts connotes; *alv*: alveolar, *c*: cardiac, *inh*: inhaled, *bro*: bronchioles, *muc*: mucosa, *art*: arterial, *b:a*: blood:air, *w:a*: water:air, *t*: tissue.

The increased blood flow to leg muscle during bicycle exercise is reflected by modeling resting muscle and skin, and working muscle separately.[99] Production of endogenous acetone, as well as acetone metabolism is included in the liver.

4.3.2 Toluene (Paper IV)

The toluene PBPK model include compartments for lungs and arterial blood, well perfused tissues, fat, muscles, and liver (Figure 6a).[95] The fat compartment is split in subcutaneous and perirenal fat, and resting and working muscles are modeled separately.[99] Metabolism occurs in both liver and lungs (i.e. the lung and arterial blood compartment).[100] The model is calibrated in a statistical framework using experimental data from 6 subjects exposed to 80 ppm of toluene for four consecutive 30-min periods during rest and exercise at increasing workloads of 50, 100 and 150 W.[101,102] The experimental data include frequently recorded levels of toluene in arterial blood, end-exhaled air and subcutaneous fat tissue.

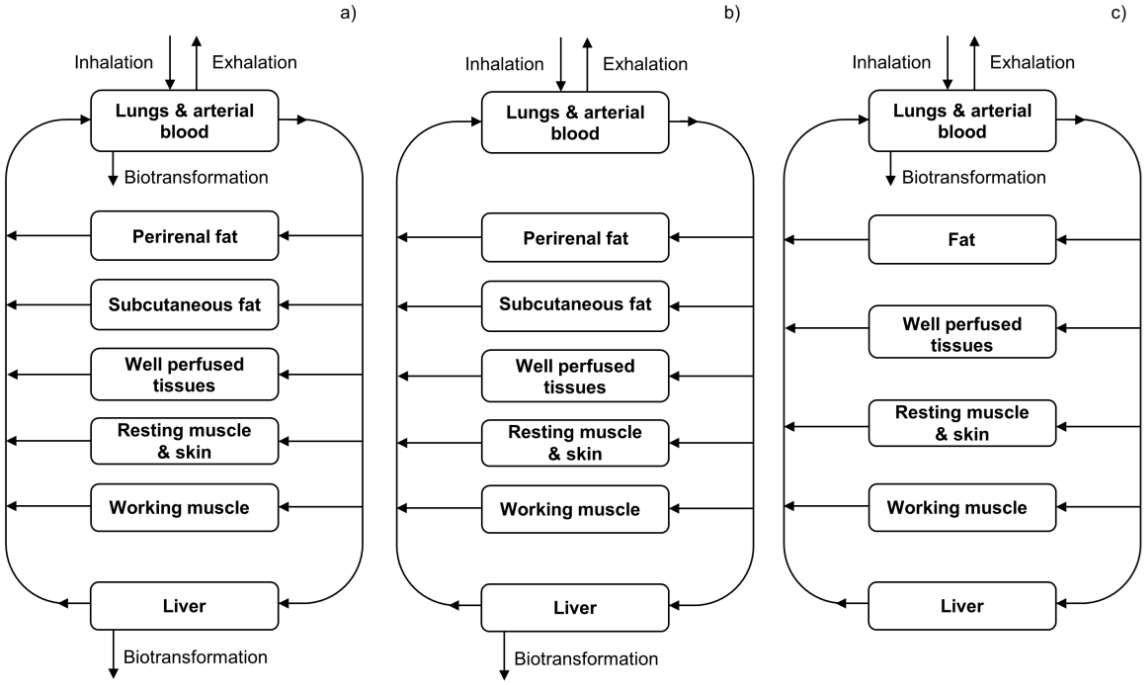

Figure 6. PBPK model structures for a) toluene, b) styrene and c) methyl chloride.

4.3.3 Styrene (Paper IV)

The styrene PBPK model include compartments for lungs and arterial blood, well perfused tissues, subcutaneous fat, perirenal fat, working muscles, resting muscles, and liver (Figure 6b).[103] Saturable metabolism is described in the liver. The styrene PBPK model is calibrated to experimental data in a hierarchical Bayesian framework. It was fitted to measured levels of styrene in arterial and venous blood, subcutaneous fat and end-exhaled air recorded from 24 individuals from three different exposure studies. The

volunteers were exposed during 120 minutes at styrene levels of between 50 and 350 ppm at rest or while performing physical activity at workloads of up to 150 W.[104-106]

4.3.4 Methyl chloride (Paper IV)

The methyl chloride population PBPK model include six compartments; lungs and arterial blood, well perfused tissues, working muscle, resting muscle, fat and liver (Figure 6c).[107] The model was developed for non-conjugators and hence elimination was originally only by exhalation. The model is calibrated to observed levels of methyl chloride in arterialized capillary blood and exhaled air from 8 individuals exposed to 10 ppm of methyl chloride vapor during 120 minutes while performing light physical exercise (50 W).[108] In this thesis, a clearance term was added to the PBPK model to account for metabolism of conjugators (Paper IV).[58,93-95,109] The experimentally estimated clearance exceeds liver blood flow for both fast and slow conjugators.[58] Thus, metabolism was for simplicity assumed to occur in blood (i.e. the lung and arterial blood compartment).

4.4 PBPK MODEL PARAMETERIZATION

4.4.1 Experimentally measured parameters (Papers I and II)

Some of the model parameters were measured in the experimental studies of exposure to acetone and were hence adopted in the PBPK model for acetone.[97,98] In both studies, body weight (BW), body height (BH), pulmonary ventilation rates and endogenous acetone levels (unpublished data) were measure. In addition, the individual exposure levels were measured throughout the exposure (unpublished data). Oxygen uptake and respiratory frequency were measured in one of the studies.[98] The individual model parameters are given in Paper I, Table 2 and Paper II, Table 2.

4.4.2 Scaled model parameters (Papers I, II and III)

To account for known physiological relationships between parameters the acetone PBPK model parameters were as far as possible scaled to covariates. Organ volumes were scaled to BW and BH. The rate of exchange of acetone via the mucosa is scaled to alveolar ventilation rate and cardiac output. The scaling of the remaining model parameters differs between the three published papers on acetone and is described below.

Paper I

Pulmonary ventilation rates were determined by experimental data. The alveolar ventilation rate was calculated from the pulmonary ventilation rate by subtracting the product of breathing frequency and dead space. Alveolar ventilation was in turn used to calculate the cardiac output by using the perfusion over ventilation ratio (PVR). Subsequently, organ blood flows were calculated as fractions of the cardiac output based on values for organ blood flows reported by Åstrand.[37] Acetone metabolism was scaled to $BW^{0.75}$ and described by Michaelis-Menten kinetics (saturable metabolism). The scaling is described in detail in Paper I (Tables 2 and 3).

Papers II and III

Blood flows were scaled to BW and BH. Cardiac output was calculated as the sum of the contributions from each organ, and was linked to alveolar ventilation via the PVR. Alveolar ventilation was in turn related to pulmonary ventilation by adding dead-space ventilation. The physiological changes during physical activity was considered proportional to the excess oxygen uptake above rest.[110] The change of tissue perfusion with physical exercise was described by equations previously derived by Droz and coworkers[111] and based on reference values suggested by Åstrand.[37] The metabolism was scaled to BW and BH, assumed to be first order and described by intrinsic clearance. The scaling is described in detail in Paper II, Table 1 and Paper III, Table 2.

4.4.3 Prior distributions (Paper II)

The compartment volumes, the volume of the dead space and the perfusion over ventilation ratio were considered known with precision. Prior distributions were defined for the remaining model parameters by complying with the philosophy of information gathering in Bayesian statistics, thus incorporating new information based on current knowledge. Thus, the prior distributions used as starting points for calibration of the acetone PBPK model were mainly derived from posteriors from the previously calibrated PBPK model for styrene.[103] Distribution of the parameters governing the washin-washout effect and the acetone chemical-specific model parameters were defined based on the summary of available evidence in the literature. Details on the derivation of prior distribution are given in Paper II (Tables 3 and 4 and the Appendix).

4.5 HIERARCHICAL MODEL (PAPER II)

The statistical model used in the calibration of the acetone PBPK model had two levels; one representing the volunteers participating in the experimental studies and one representing the population. It is schematically represented in Figure 7. At the individual level, each volunteer is represented by the measured parameters (φ) (e.g. exposure level, BW, BH oxygen uptake and lung ventilation rates) and the observed concentrations of acetone blood and breath over time. The remaining, unknown individual parameters (θ) are considered samples from a population distribution and were defined by prior distributions. The PBPK model can predict the time-concentration profile for an individual given φ and θ. The prediction is then compared with the observations. They will differ due to errors in experimental measurements, and in model- or parameter specification. Thus, errors were defined for each experimental biomarker (arterial- and venous blood and mixed- and end-exhaled air) while assuming that the errors were independent, identical in the two experimental studies and log-normally distributed with mean 0 and variance σ^2. As μ and Σ, as well as σ^2 are not known with precision they were considered associated with uncertainty and hence expressed by probability distributions. Lognormal distributions were chosen for the population means (μ) while inverse gamma distributions were used for the population variability (Σ),[112] and error σ^2.[113,114]

4.6 MARKOV CHAIN MONTE CARLO SIMULATION (PAPER II)

The calibration of the acetone PBPK model to the human experimental toxicokinetic data was performed via MCMC simulation using Metropolis-Hastings sampling.[74] The simulations were performed in the McSim software.[115] Convergence was assessed in the Bayesian Output Analysis Program (BOA),[116] using the criterion of Gelman and Rubin,[73] and by visual inspection of the chains.

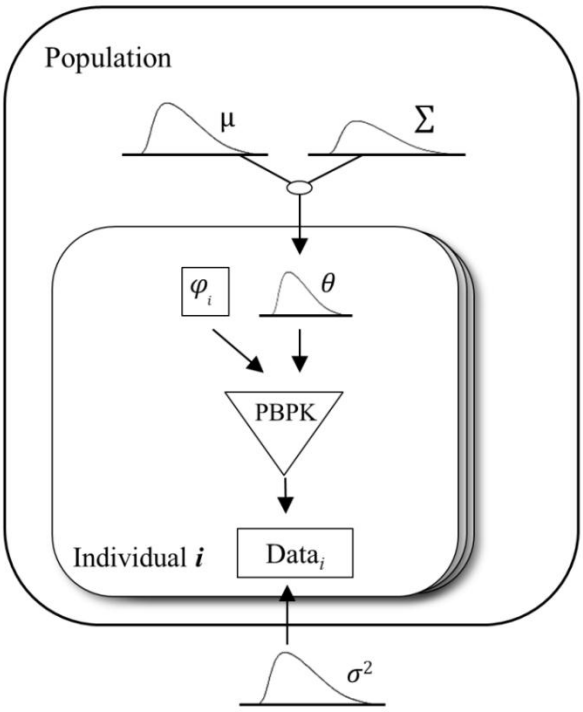

Figure 7. The hierarchical model used in the calibration of the PBPK model for acetone. The PBPK model can predict the concentrations of acetone for each individual (i) from the experimentally measured parameters (φ) and unknown parameters (θ). The unknown parameters are defined at the population level by their mean (μ) and variability (Σ). The deviation between the model predictions and the data is represented by σ^2.

4.7 MONTE CARLO SIMULATIONS (PAPERS III AND IV)

4.7.1 Dose metrics

The amount of parent compound or metabolite in blood (or other biomarkers), i.e. the surrogate dose, is generally thought to be a good indication of the internal dose. The choice of surrogate doses for acetone, toluene, styrene and methyl chloride was based on information about the toxic entity of each solvent (section 4.1).

Markers for chronic effects

The surrogate doses for chronic effects from exposure to acetone and toluene were the 24 hour integrated concentration (AUC_{24h}) of parent compound in blood. For styrene and methyl chloride the metabolized amount during 24 hours, normalized to $BW^{0.75}$ (Met_{24h}) was chosen.

Markers for acute effects
Acute effects for all four chemical substances were represented by the maximal concentration of parent compound in blood (C_{Max}).

4.7.2 Exposure scenarios

General population
Monte Carlo simulations were performed in 8 different sub-populations; 3 month old babies, 1 year, 5 year and 10 year old children, 15 year old males and females, and adult males and females. The individuals were assumed to be continuously exposed to solvent levels air corresponding to the set or suggested RfC while performing physical activity according to reference values for daily time budgets, specific for each subpopulation.[117]

The impact of endogenous acetone levels could be important during low dose exposures. Therefore, the simulations in the general population both included and excluded endogenous acetone.

The outcome of 10 000 MC simulations was collected for each sub-population and activity level. The simulations were performed using the McSim software.[115]

Workers
The male and female workers were assumed to be exposed to the TLVs recommended by ACGIH, from 8 am to 12 pm, and from 1 pm to 5 pm. During lunch (12 am to 1 pm) and from 5 pm to 8 am the next morning the exposure was set to 0 ppm. Light physical exercise was assumed during the morning and afternoon shifts. During lunch, from 5 pm to 11 pm and between 7 am and 8 am the workload was half of what was applied during the shifts. During night (11 pm to 7 am) the workers were resting. The influence of fluctuating exposure and workload, and workplace ventilation rate on the surrogate doses was investigated by simulating six different exposure scenarios;

- Constant exposure and constant workload
- Constant exposure and fluctuating workload
- Fluctuating exposure with slow air exchange, and constant workload
- Fluctuating exposure with rapid air exchange, and constant workload
- Fluctuating exposure with slow air exchange, and fluctuating workload
- Fluctuating exposure with rapid air exchange, and fluctuating workload

The outcome of 10 000 MC simulations was collected for each gender and exposure scenario. The simulations were performed using the McSim software.[115]

4.7.3 Distributions of the model parameters

The parameter distributions representing BW, body mass index (BMI), oxygen uptake and BH were derived from vital statistics or the scientific literature. Remaining parameters distributions were defined by the posteriors from previous calibrations of the acetone, toluene, styrene and methyl chloride PBPK models (used mainly for adult males),[97,101,105,115] and model parameter distributions collected from the scientific literature (used mainly for adult females and children).

4.7.4 Calculation of $CSAF_{HK}$ (Papers III and IV)

The chemical specific adjustment factors for human toxicokinetic variability ($CSAF_{HK}$) were derived based on the 90^{th}, 95^{th} and the 97.5^{th} percentile of simulated surrogate doses in a given population.[21] Hence, they are considered to protect 90, 95 and 97.5 percent of the individuals in the population. $CSAF_{HK}$ were derived by dividing the n^{th} (where n is 90, 95 and 97.5) percentile of surrogate doses in each sub-population, with the median surrogate dose in the whole population. The distribution of surrogate doses in whole general population was obtained by collecting surrogate doses from each sub-population while considering its proportion of the Swedish population.[118] The surrogate doses representing the whole worker population were calculated in a similar manner by collecting those obtained by simulating constant exposure and constant workload, while considering the proportion of females in industry with potential exposure to acetone (26% of workers), toluene (19%), styrene (26%) and methyl chloride (6%).[2] For methyl chloride, the proportion of the Swedish population belonging to each GSTT1 genotype was also considered (11.1% null; 46.2% slow; and 42.8% fast conjugators).[119] In the calculation of $CSAF_{HK}$ for methyl chloride, the fast and non-conjugators were considered the most sensitive group for chronic and acute effects, respectively.

5 RESULTS

5.1 PBPK MODEL FOR ACETONE (PAPER I)

A washin-washout PBPK model for acetone (described in Section 4.3.1) was developed in Paper I. The model predictions were compared to those obtained by using an inert tube PBPK model. The wasin-washout model allows pre-alveolar utake of inhaled acetone, whereas the the conducting airways of an inert tube model are assumed only to transport the solvent vapor to the alveoli, where all gas exchange occurs (an example of an inert tube model is depicted in Figure 3). The washin-washout model was able to accurately predict average acetone levels in blood and breath sampled from 16 inhalation experiments with 8 male volunteers exposed to acetone levels ranging from 250-550 ppm, during various levels of physical activity. In contrast, the inert tube model was unable to describe the experimental data, especially in exhaled air (Figure 8).

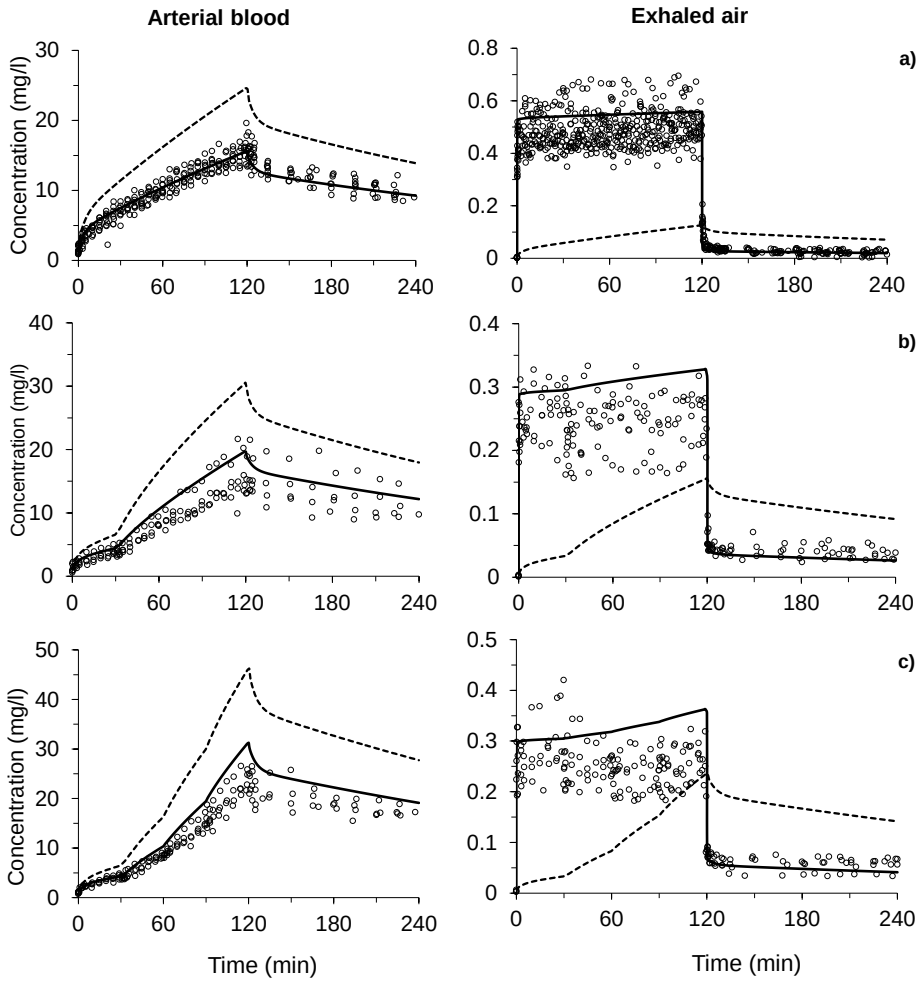

Figure 8. Washin-washout (solid lines) and inert-tube model (dotted lines) predictions are compared to experimental acetone concentrations (circles) in arterial blood and exhaled air. **a)** Exposure to 540 ppm for 120 minutes during resting conditions. **b)** Exposure to 290 ppm for 30 minutes at rest followed by 90 minutes of physical exercise at 50 W workload. **c)** Exposure to 290 ppm for 30 minutes at rest followed by a stepwise increase in workload (50, 100, 150 W) for 30 minute periods.

To evaluate the predictive power of the finalized acetone washin-washout PBPK model it was applied on a new dataset which was withheld during the model specification. The predictions of acetone in arterial blood and mixed exhaled air agreed well with the observed levels of acetone (Figure 9)

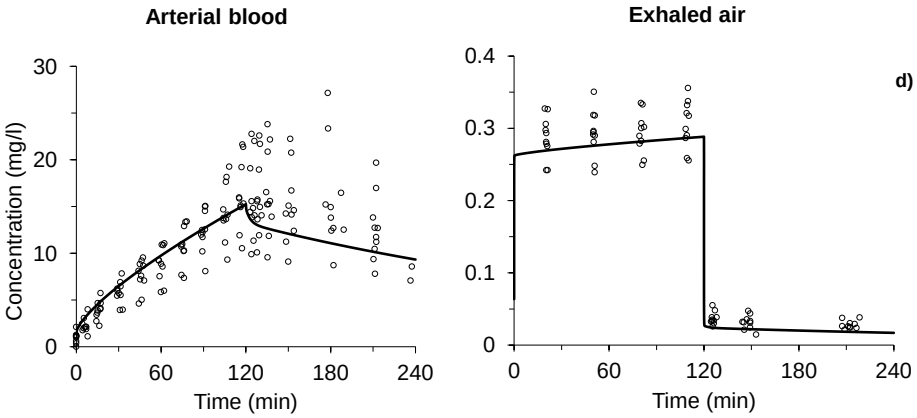

Figure 9. Predictive power of the washin-washout PBPK model. The prediction of average acetone levels in blood and breath is compared to observed levels from 10 male volunteers exposed during 120 minutes to 250 ppm of acetone during the performance of physical exercise at 50W workload.

5.2 ACETONE PBPK MODEL CALIBRATION (PAPER II)

In Paper II, the washin-washout PBPK model for acetone was calibrated to experimental toxicokinetic data from 18 male volunteers exposed to 250-550 ppm of acetone while performing physical activity (section 4.2) in a Bayesian hierarchical framework using MCMC simulation. This was done to obtain improved estimates of population variability and uncertainty of the PBPK model parameters, especially for those related to the washin-washout behavior of polar volatile substances. Meanwhile, the sensitivity of PBPK model to the prior assumptions was investigated.

5.2.1 Sensitivity analysis

The sensitivity analysis indicated that for most physiological model parameters no new information on population variability could be obtained from the experimental toxicokinetic data. Thus, in subsequent MCMC simulations population variability was estimated only for clearance and the parameters governing the washin-washout of acetone in the airways.

5.2.2 Posterior distributions

Following the calibration of the acetone PBPK model to the experimental data, the precision of most of the population parameter estimates was improved. Moreover, most parameters were found to be uncorrelated (Paper II, Table 4). New information was particularly gained on the population distribution of the parameters governing the washin-washout effect. The population mean and variability of the model parameters are listed Table 1, along with the relative change of the posterior estimates compared to the prior distributions.

Table 1. Posterior distributions of the acetone PBPK model parameters summarized by the population mean (μ) and variability (Σ), with associated uncertainties, UC (coefficient of variation). The percent change from the prior distribution is also indicated. A negative sign implies that the precision of the posterior estimate was improved compared to the prior.

Acetone PBPK model parameter		Posterior distribution		Change from prior (%)	
		μ^a (UC_M)	Σ^b (UC_V)	$\mu(UC_M)$	$\Sigma(UC_V)$
Unit perfusion (l/(min · l tissue))					
QC_{Fat}	Fat	0.02 (0.11)	-	0 (1)	-
QC_{Rpt}	Richly perfused tissues	0.75 (0.05)	-	- 6 (-5)	-
QC_{Mus}	Muscle and skin	0.04 (0.01)	-	33 (-9)	-
QC_{Liv}	Liver	0.85 (0.08)	-	- 3 (-2)	-
Perfusion change with exercise (l/(min · l tissue) / (l O_2/min))					
QC_{Fat}^{Work}	Fat	0.01 (0.22)	-	0 (-4)	-
QC_{Rpt}^{Work}	Richly perfused tissues	0.22 (0.05)	-	22 (-5)	-
QC_{Wm}^{Work}	Working muscle	6.91 (0.01)	-	-18 (-12)	-
QC_{Liv}^{Work}	Liver	0.14 (0.12)	-	8 (-1)	-
Metabolism (l/(min·l liver))					
C_L	Clearance	0.07 (0.07)	0.37 (0.24)	0 (-2)	11(14)
Washin-washout					
QC_{BroMuc}	Fractional transfer between bronchioles and mucosa	0.89 (0.06)	0.30 (0.42)	11 (-4)	0 (-158)
QC_{MucArt}	Fractional transfer between mucosa and arterial blood	0.74 (0.10)	1.00 (0.10)	-8 (0)	70 (-190)
Partition coefficients					
P_{FatA}	Fat:air	82 (0.11)	-	-5 (-5)	-
P_{RptA}	Richly perfused tissues:air	208 (0.02)	-	52 (-26)	-
P_{MusA}	Muscle:air	195 (0.02)	-	29 (-47)	-
P_{LivA}	Liver:air	192 (0.07)	-	14 (-43)	-
P_{BA}	Blood:air	221 (0.00)	-	-22 (-11)	-
P_{WA}	Water:air	354 (0.08)	-	1 (-1)	-

[a] arithmetic mean

[b] coefficient of variation of the mean

5.2.3 Validation

To validate the calibrated PBPK model, the experimentally observed acetone concentrations in arterial- and venous blood and end- and mixed exhaled air were simulated by using the PBPK model, the experimentally determined model parameters and subject-specific posterior estimates. In general, the predictions agreed well with the observed concentrations (Figure 10). However, the predictions of acetone levels in breath at low concentrations were not as precise as the other predictions (Figure 10 b and d).

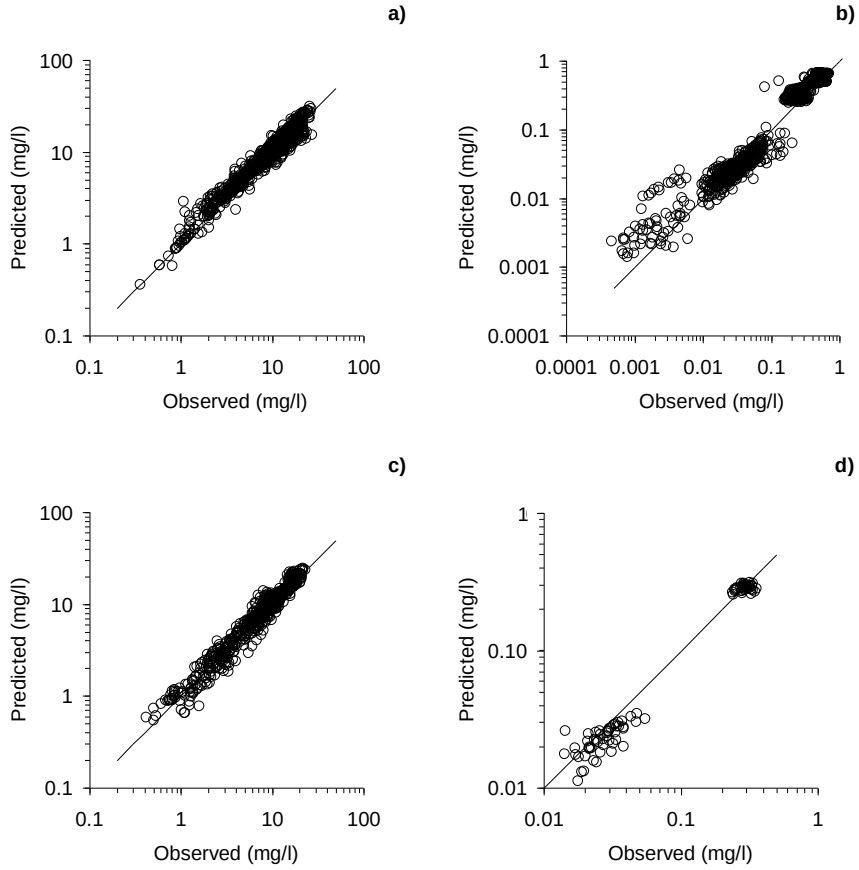

Figure 10. Predicted versus observed acetone concentrations in **a**) arterial blood, **b**) end-exhaled air, **c**) venous blood, and **d**) mixed exhaled air.

5.3 HUMAN TOXICOKINETIC VARIABILITY (PAPERS III AND IV)

$CSAF_{HK}$ for acetone, toluene, styrene and methyl chloride were derived by Monte Carlo simulation, combining PBPK models for respective solvent, population distributions of the model parameters from previous Bayesian analyses, vital statistics and published distributions of physiological and anatomical parameters for adult females and children. The simulations covered how factors such as age and gender in the general population, and fluctuations in workplace air concentration and workload influenced the surrogate doses in adult female and male workers. For acetone, the influence on the surrogate doses by endogenous production was also investigated. Chronic effects of solvent exposure were considered important in the general population as well as for workers. In workers, acute effects of solvent exposure were also explored. $CSAF_{HK}$ were calculated for the 90^{th}, 95^{th} and the 97.5^{th} percentiles of simulated surrogate doses in each population as a whole, in each sub-population and for each exposure scenario. The $CSAF_{HK}$ reported below were derived at the most commonly used 95^{th} percentile, thus considered to protect 95 percent of the individuals in the population. The factors at the other percentiles were marginally different. The $CSAF_{HK}$ for acetone are tabulated in Paper III (Tables 6, 7 and 8). The $CSAF_{HK}$ for toluene, styrene and methyl chloride are tabulated in Paper IV (Appendices A and B).

5.3.1 Acetone

General population

In the whole population, the intraspecies differences of surrogate dose were accounted for by a $CSAF_{HK}$ of 1.9 (Figure 11 and Paper III, Table 6). However, endogenous acetone levels had a significant influence on the predicted surrogate doses. Thus, a factor 2.9 was obtained when including variability from endogenous sources (Paper III, Table 6). Higher $CSAF_{HK}$ were obtained in children. Hence, factors up to 2.4 and 5.5 (including endogenous acetone) were derived. The $CSAF_{HK}$ obtained for males were slightly higher compared to the ones derived for females.

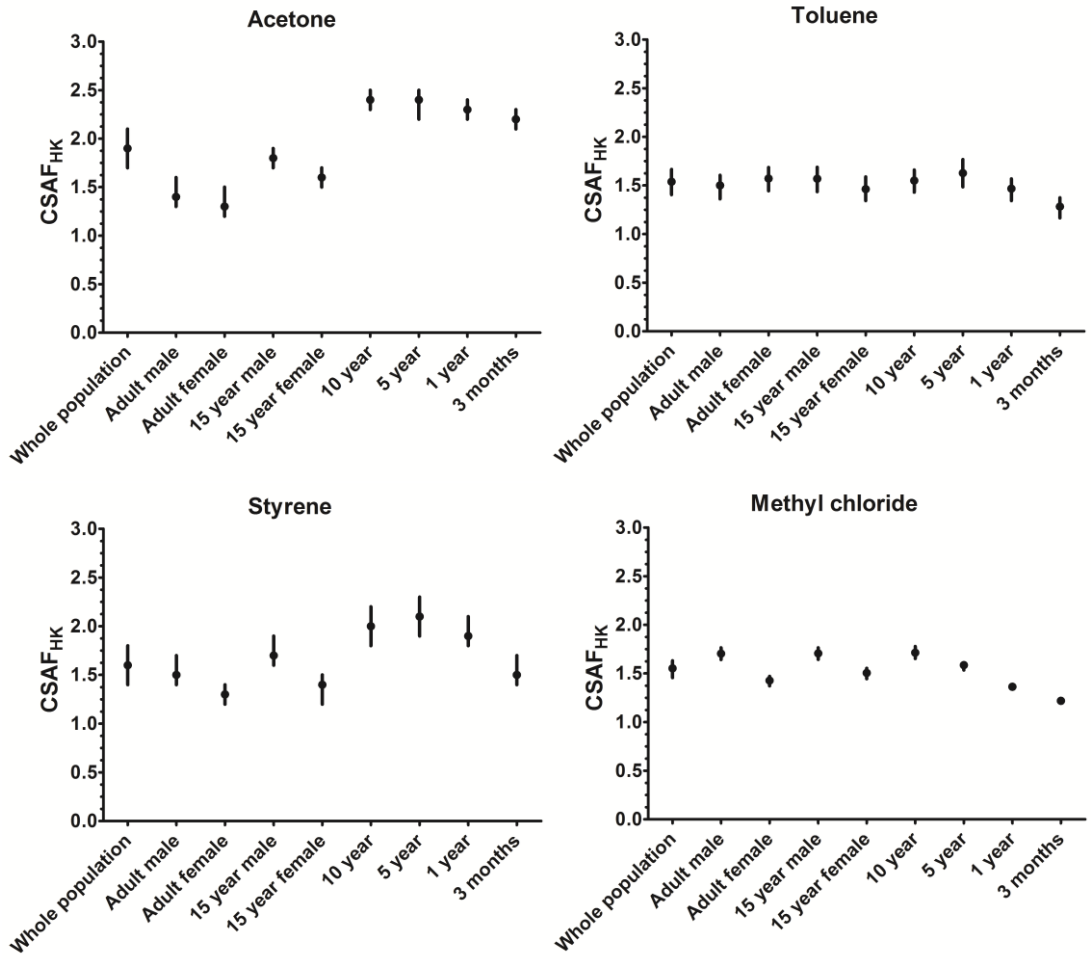

Figure 11. $CSAF_{HK}$ for acetone, toluene, styrene and methyl chloride for chronic effects in the general population. The $CSAF_{HK}$ for acetone excludes variability from endogenous sources. The $CSAF_{HK}$ for methyl chloride represent sub-populations homozygous for GSTT1 (i.e fast conjugators), thus having the highest metabolism in the population.

Workers

During conditions of constant exposure and workload, $CSAF_{HK}$ of 1.5 (chronic) and 1.3 (acute) were derived for the entire workforce (Figures 12 and 13). The influence of fluctuating exposure or workload on the derived $CSAF_{HK}$ was small. However, the combined effect of fluctuations in both exposure and workload increased the $CSAF_{HK}$,

especially during conditions of rapid air exchange (up to 1.8). Gender differences were negligible (Paper III, Tables 7 and 8).

5.3.2 Toluene

General population

Considering the general population as a whole, a factor of 1.5 was calculated to cover the intraspecies variability of toluene (Figure 11 and Paper IV, Appendix A). The $CSAF_{HK}$ derived for children were slightly higher (up to a factor 1.6). Gender differences in the toxicokinetics of toluene were small. Thus, 15 year old males had slightly higher factors than 15 year old females. The factors for adult females were slightly higher compared to the ones for adult males.

Workers

The variability of surrogate doses representing both chronic and acute effects of toluene was covered by a factor 1.6, considering the whole workforce during conditions of constant exposure and constant workload (Figures 12 and 13). When fluctuations in either exposure or workload were applied, the $CSAF_{HK}$ increased slightly. Combining fluctuations in exposure and workload further increased the factors. Slight gender differences among workers were observed. Thus, female workers had consistently higher $CSAF_{HK}$ than male workers. Under the most extreme assumptions of fluctuating workload and fluctuating exposure the factors were 2.0 (chronic) and 3.5 (acute) (Paper IV, Appendix B). The rate of air exchange affected the $CSAF_{HK}$ for acute effects. Thus the factors were higher during conditions of rapid compared to slow air exchange (Figure 13).

5.3.3 Styrene

General population

Intraspecies toxicokinetic variability of styrene was represented by a factor of 1.6 in the whole population (Figure 11, and Paper IV, Appendix A). According to the simulations, the influence factors increased up to 2.1 when including the effect of age. Males had slightly higher $CSAF_{HK}$ than females.

Workers

The $CSAF_{HK}$ for styrene were 1.7 (chronic) and 1.8 (acute), considering the whole worker population and conditions of constant exposure level and constant workload (Figures 12 and 13). Fluctuations in exposure level or workload increased the factors. Further increases were obtained when combining both types of fluctuations. The $CSAF_{HK}$ representing chronic effects were higher for male than female workers. Conversely, female workers had higher factors for acute effects than males. Hence, the highest factors in our simulations were 2.0 (chronic) and 4.7(acute) (Paper IV, Appendix B). The rate of air exchange did not markedly affect the factors derived for chronic effects. However, rapid air exchange markedly increased the $CSAF_{HK}$ for acute effects (Figure 13).

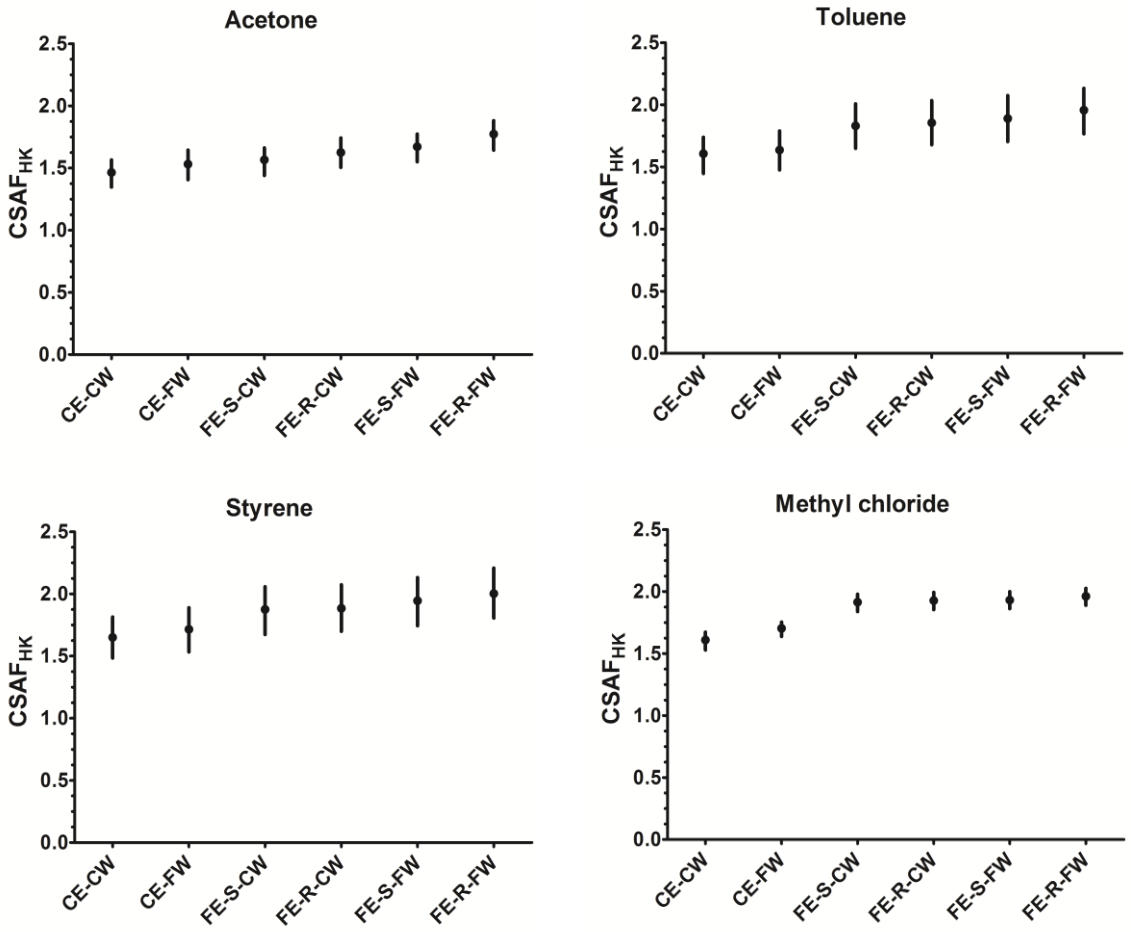

Figure 12. $CSAF_{HK}$ for chronic effects in workers during various workplace exposure scenarios. The factors for methyl chloride represent workers homozygous for GSTT1 (i.e fast conjugators), thus having the highest metabolism in the population. Abbreviations are C; constant, E; exposure level, F; fluctuating, R; rapid air exchange and S; slow air exchange, W; workload.

5.3.4 Methyl chloride

General population

A factor of 1.6 covers intraspecies toxicokinetic variability in the general population when considered as a whole (Figure 11 and Paper IV, Appendix A). Age had a slight influence the $CSAF_{HK}$. Adult males had slightly higher surrogate doses than adult females. The same difference was detected for 15 year old males as compared to 15 year old females.

Workers

Among workers, $CSAF_{HK}$ of 1.6 (chronic) and 1.3 (acute) were derived during constant exposure and constant workload (Figures 12 and 13). Fluctuations in workload had only a slight effect on the derived $CSAF_{HK}$ whereas fluctuations in exposure level enhanced variability in surrogate dose. The factors were further increased by a combination of fluctuations in exposure level and workload. Male workers had higher

$CSAF_{HK}$ for chronic effects whereas females had slightly higher factors based on acute markers of effects. Hence, the highest derived $CSAF_{HK}$ in the simulations were 2.0 (chronic) and 6.1 (acute). The $CSAF_{HK}$ derived for acute effects were increased markedly during conditions of raid air exchange (Figure 13).

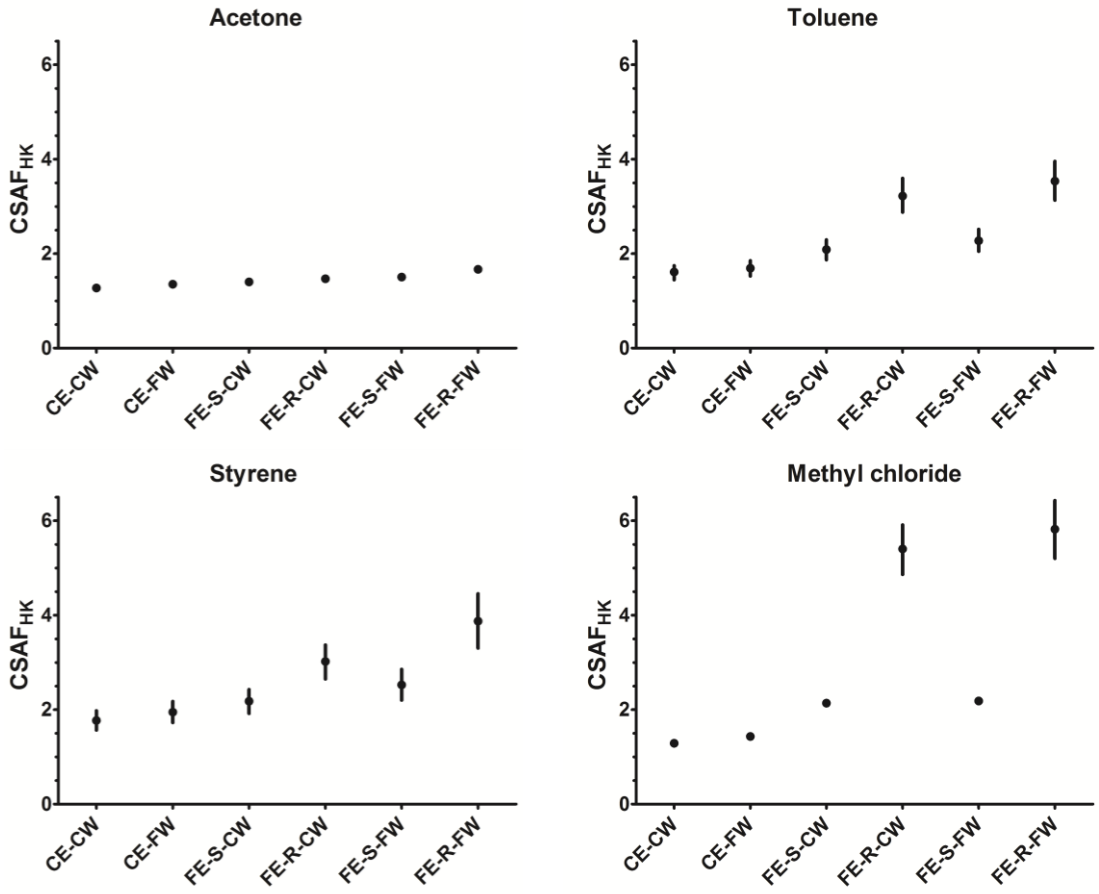

Figure 13. $CSAF_{HK}$ for acute effects in workers during various workplace exposure scenarios. The factors for methyl chloride represent workers with no conjugation (null genotype), thus having the highest blood concentrations of methyl chloride in the population. Abbreviations are C; constant, E; exposure level, F; fluctuating, R; rapid air exchange and S; slow air exchange, W; workload.

6 DISCUSSION

The overall aim of this thesis was to improve the scientific basis for human health risk assessment factors. It was achieved by developing a probabilistic framework for the derivation of chemical specific assessment factors describing intraspecies differences in the toxicokinetics ($CSAF_{HK}$) of inhaled solvent vapors. The method uses PBPK modeling in combination with Monte Carlo simulation from population distributions of physiological and biochemical model parameters to simulate surrogate doses in the general and worker populations. The effects of age, gender and various workplace conditions on the surrogate doses were investigated. $CSAF_{HK}$ were derived for four commonly used organic solvents; acetone, toluene, styrene and methyl chloride.

6.1 PROBABILISTIC FRAMEWORK

As in any modeling exercise, the findings presented in this thesis are influenced by the assumptions and considerations made during the development, calibration and parameterization of the population PBPK models for acetone, toluene, styrene and methyl chloride. They are discussed in detail in the following sections.

6.1.1 PBPK models

Four different PBPK models were deployed in the derivation of the $CSAF_{HK}$. The acetone PBPK model was developed and calibrated in Papers I and II. PBPK models for toluene, styrene and methyl chloride were already described in the scientific literature and were hence adopted in the MC simulations in Paper IV. The PBPK models have many features in common, but there are also differences. The ones considered to be the most influential are discussed below;

Washin-washout effect

As shown in Paper I, an adequate specification of the inhalatory uptake is important to correctly estimate the internal dose of hydrophilic volatiles. The uptake of polar solvents such as acetone is largely affected by the influence of washin-washout of solvent vapor in the respiratory airways during inhalation and exhalation. The uptake of less polar solvents, such as styrene, toluene and methyl chloride, may only be slightly or not affected at all. Washin-washout of solvent vapor is described in the PBPK models for acetone and styrene. A set of correction factors used to reduce alveolar ventilation were sufficient to address the washin-washout of styrene.[103] The correction factors were determined by simultaneous fitting to observed levels of styrene in arterial blood and end-exhaled air. In contrast, the washin-washout of acetone (Paper I) was described by dividing the lung into 4 separate compartments, and by allowing acetone to be exchanged with systemic blood in both the alveolar region and bronchioles. Various mathematical descriptions of the washin-washout effect have previously been attempted for acetone, but none have succeed in reproducing observed levels of solvent in both blood and breath at elevated levels of physical activity.[15,17,127-129] The acetone PBPK model developed in Paper I enabled simultaneous descriptions of acetone levels

in arterial blood, and end- and mixed exhaled air, during levels of physical activity up to 150W (Section 5.1, Figure 8).

Physical activity

Exposure to solvent vapors often occurs while performing various tasks requiring physical activity, in the workplace as well as in residential settings. Thus, simulations of internal doses used to derive $CSAF_{HK}$ were performed by including physical activity for the general population as well as for workers. For acetone, workloads of 50W and 150W for light and heavy exercise respectively were used for all sub-populations (Paper III). For toluene, styrene and methyl chloride, the workloads were instead determined by matching lung ventilation to reference values for light and heavy exercise in each sub-population (Paper IV). The perfusion change during physical exercise was accounted for in a similar manner in all four models. To allow for differences in blood flow to working and resting muscles, the muscle compartment is divided into two.[99] Similarly, the fat compartment of the toluene and styrene PBPK models is divided in two to represent the differences in blood flow to subcutaneous and perirenal fat during physical exercise.[95] The change of the tissue perfusion rates is determined by the excess oxygen uptake above rest (ΔVO_2) and the workload, except for the perfusion change of perirenal fat which was set to the same elevated level during all exercise levels.[116,117,95] According to the model scaling equations adopted from Droz and coworkers,[111] the change of perfusion during physical exercise of well perfused tissues, liver and fat is related to BW and BH. However, the increase of the blood flow to working muscle is described independent of body size (Paper III, Table 1 and Paper IV, Table 1).

Perfusion over ventilation ratio

The perfusion-ventilation ratio *(PVR)* relates lung ventilation to cardiac output. Thus, it is a key parameter in the inhalation PBPK models described in this thesis. In theory, the PVR equals 1. However in vivo, it varies between different parts of the lung and depends on the position the body is in.[120] When the PVR differs from 1 inequality or mismatch between perfusion and ventilation occurs. Mismatch has been observed during physical exercise,[121] especially during high workloads.[122,123] Inequality has also been suggested in young children as the lung continues to develop until the age of approximately 8 years.[124] However, there is lack of experimental data on the PVR in the scientific literature, especially for healthy adult females and children. There is also a large variation in the values used in previous models (Paper IV, the Discussion). Due to the lack of consistent information, the PVR is one of the most uncertain parameters in the simulations.

Metabolism

In Papers II and III, acetone metabolism was assumed to be first order and described by a single clearance parameter. This contrasts the Michaelis-Menten metabolism assumed in Paper I, and in the other previous PBPK models for acetone.[125,126] The reason for using a linear model is that the metabolic parameters V_{Max} and K_m could not be estimated separately in the Bayesian calibration process due to a high correlation

between the two (Paper II). The assumption of linear kinetics will have negligible impact on the estimates of toxicokinetic variability during exposure to low levels of acetone. However, at high exposure levels, such as the ones in an occupational setting, metabolism could be partially saturated. Thus, the assumption of linearity had the potential to affect the $CSAF_{HK}$. Additional MC simulations using Michaelis-Menten metabolism concluded that the impact of metabolic saturation on the $CSAF_{HK}$ for acetone derived for workers was negligible.

The PBPK model for methyl chloride was originally developed for non-conjugators and hence did not include metabolism. In Paper IV metabolism was added to the model to include also slow and fast metabolizers of methyl chloride. Metabolism was assumed to be linear, to occur in blood and was based on human experimental data from adult subjects. These choices may influence the $CSAF_{HK}$ for chronic effects, but the effects on the $CSAF_{HK}$ for acute effects are expected to be minimal. Those were derived based on surrogate doses from non-conjugators, i.e. individuals with no metabolism of methyl chloride of methyl chloride.

Predictive power

The predictive power of a PBPK model may be assessed by applying it to a new data set which was not used during model specification. Such a procedure can avoid dictation of the choice of model structure or parameterization by the experimental data, i.e. over fitting of the PBPK model.[45,127] Ideally, the available experimental data would be divided in two sets - one set which is available during development and refinement of the PBPK model and one which is withheld during this phase, and thus may represent new data to which the predictions of the finalized PBPK model can be compared. In the case of acetone, splitting of the data was performed during the model development phase (Paper I). However, to propagate as much information as possible to the posterior population distributions of the model parameters, all the experimental data were subsequently deployed in the calibration of the acetone PBPK model (Paper II). The predictive power of the toluene, styrene and methyl chloride PBPK models was not assessed.[95,103,107]

6.1.2 PBPK model calibration

Calibration of PBPK models to experimental data is essential if the outcome of the modeling activities is to be used in health risk assessment of chemical substances.[65] Obviously, calibration is dependent on the availability and nature of the experimental data. The PBPK models for acetone, toluene, styrene and methyl chloride were individually optimized in Bayesian frameworks using MCMC simulation. During a Bayesian analysis, prior knowledge on the population distributions of the model parameters is increased by adding information contained in experimental toxicokinetic data.

Prior knowledge

Knowledge and experience is individual. Therefore, different persons may draw different conclusions from previous knowledge, and thus use different prior

distributions. Hence, it has been argued that the Bayesian approach is subjective. The idea of a PBPK model is to use biological knowledge in the model specification and parameterization. Therefore, published literature values used to derive the prior distributions used as starting points for the Markov chains. The reference literature was approached in an objective fashion by trying to include all available values while giving priority to human in vivo data. In order to comply with the philosophy of information gathering in Bayesian statistics, thus incorporating new information based on current knowledge, the prior distributions for the physiological model parameters were derived from the posteriors resulting from previously calibrated models, when available. By this procedure, it is assumed that knowledge on the physiological model parameters is fully taken advantage of.

Experimental toxicokinetic data

The experimental toxicokinetic data for acetone, styrene, toluene and methyl chloride is very detailed, i.e. it includes frequent sampling of levels of a chemical substance in several biomarkers. However, it comes from a limited number of relatively homogenous individuals. The use of such data in PBPK modeling for human health risk assessment purposes has both pros and cons. The detailed information offers possibilities to gain valuable knowledge on the biological processes that determine the toxicokinetics of chemical substances, and hence partially the risk of adversity. However, it is not certain that the conclusions are valid for larger and more heterogeneous populations. Obviously, experimental studies which include a larger number of more diverse volunteers would be helpful in resolving this issue. Such studies are however costly and also ethically questionable. However, chemical-specific toxicokinetic information from several already conducted experiments can be combined by Bayesian population analysis and thus help reduce such uncertainty. The experimental data used for calibration of the acetone (Paper II), toluene, styrene and methyl chloride (performed in the original studies) PBPK models was derived from a total of 56 volunteers, whereof 4 were female. Hence, the experimental data offered limited opportunities to access possible age or gender differences in the toxicokinetics of the four solvents. The data describe levels of solvent in venous and arterial blood, mixed and end-exhaled air during various exposure levels and several different levels of physical activity. Thus, it enables the physiological changes occurring during physical exercise to be examined. Such information is valuable when it comes to quantitative risk assessments based on PBPK modeling of internal dose. Exposure of workers, as well as of the general population to organic solvents often occurs in conjunction with physical activity.

Sensitivity to prior assumptions

To investigate the impact of the choice of prior distributions on the outcome, a sensitivity analysis was performed within the Bayesian framework during calibration of the acetone PBPK model (Paper II). Thus, four different sets of priors were constructed where 1, 10, 50 and 100 percent (CV) uncertainty was allowed to surround the prior population variability ($\sum$) of the physiological model parameters. As the information in the scientific literature regarding population variability was limited, inverse gamma

distributions were chosen. They are considered flexible enough to allow the experimental data to define variability without making too strict prior assumptions about its size.

The results of the sensitivity analysis revealed that the posterior uncertainties increased proportionally in relation to the prior. However, the posterior errors remained similar regardless of the prior set. This can be explained by a combination of factors. The population variability of the physiological model parameters in the acetone PBPK model may already to a large extent be accounted for by the scaling to BW and BH (Paper II, Table 1). Moreover, as acetone distributes rapidly and evenly in the body, the experimental data may have been be too sparse to enable the variability of the model parameters to be estimated. It is also possible that the acetone PBPK model is insensitive to some of the model parameters. Nevertheless, it seems that allowing for extensive flexibility in cases where the information content in the data is low (compared to the complexity of the model) seems to create a too large sampling space. This behavior has also previously been observed for a complex, mechanistic model.[128]

The results propose that the choice of prior distributions may have a strong influence on the posterior distributions. Thus, information on prior sensitivity may be important if the results from Bayesian analyses are to be used to estimate health risks. Formal sensitivity analyses were not performed during the calibrations of the toluene, styrene and methyl chloride PBPK models. However, various sets of priors were tested and evaluated throughout the process.[129]

6.1.3 Model parameter distributions

There is lack of experimental data from females and children, both when it comes to experimental toxicokinetic data and PBPK-relevant physiological parameters. In spite of representing a large and increasing part of the working population, females are generally less often studied than men, and gender differences are not often investigated.[130,131] However, the number of exposure studies which include female volunteers has increased in recent years.[132-135] Obvious ethical reasons limit the availability of experimental data from children. Data on physiological parameters for children were relatively scarce, especially for the youngest children included in the MC simulations. As the Bayesian calibrations were performed with experimental toxicokinetic data primarily from adult males the posterior distributions resulting from the Bayesian analyses were considered representative of adult males and hence used in MC simulations to derive surrogate doses and $CSAF_{HK}$ for adult males. The model parameter distributions for adult females and children were instead as far as possible collected from the scientific literature. If information was unavailable, the posterior distributions were used also for adult females and children. Due to the general lack of information, the $CSAF_{HK}$ derived for adult females and children are possibly associated with larger uncertainty than the ones obtained for males.

Model parameter correlations

It is well known that physiological parameters correlate. Thus, in this thesis correlations were as far as possible accounted for scaling to BW and BH. However, additional correlations may exist. In Bayesian calibration, the estimation of one model parameter is conditioned not only on the experimental data, but also on the values of all other model parameters. Thus, information on additional inter-parameter correlations is contained in the converged Markov chains. If parameter values from such chains are used as deterministic inputs to the PBPK model, the variability of internal dose may be smaller than one would anticipate from the combination of values from separate model parameter distributions. The posterior chains were available for acetone, but not for toluene, styrene and methyl chloride. For those three, summarized posterior distributions were instead used. The parameter distributions for adult female and children were in both studies mainly derived from the scientific literature. Thus, only the adult male $CSAF_{HK}$ for acetone were derived by including additional parameter correlations. The discrepancy may have resulted in a slight overestimation of the adult females and children's $CSAF_{HK}$ for acetone.

6.2 HUMAN TOXICOKINETIC VARIABILITY

$CSAF_{HK}$ for acetone, toluene, styrene and methyl chloride were derived for various sub-populations by investigating how factors such as age, gender, endogenous acetone levels, workplace ventilation rate and fluctuations in exposure levels and workload influenced toxicokinetic variability. The main findings are discussed below.

6.2.1 Age differences

Age differences were observed for acetone, toluene and styrene (Figure 11). The $CSAF_{HK}$ obtained for 5 to 10 years old children were consistently higher than the ones for adults and children of other age groups. No age differences were found for methyl chloride.

For highly and moderately blood soluble solvents such as acetone (blood; air partition coefficient, P_{BA} of 220) and styrene (P_{BA} = 64) the uptake by inhalation is mainly governed by the air flow in the lungs, however metabolism and cardiac output are also important factors.[26,33,136] Thus, the larger $CSAF_{HK}$ for acetone and styrene in children may in part be explained by children's higher oxygen demand per kg body weight (Paper IV, Table 3) and, as a consequence, higher pulmonary ventilation as compared to adults. The higher factors obtained in the 5-10 year old children are probably explained by their higher degree of physical activity compared to children of other ages, and thus relatively higher exposure by ventilation.[48] As an equilibrium between inhaled air and blood is reached more quickly for less soluble substances such as toluene (P_{BA} = 16) and methyl chloride (P_{BA} = 1.7), the effect of ventilation on the surrogate doses is less pronounced, or negligible.

The children's $CSAF_{HK}$ were up to 2.4, thus well below the default factor of 3.16 for toxicokinetic differences (Figure 11). However, for acetone, factors higher than the default were obtained when including variability from endogenous production (up to

5.5 for 3 months old children). This is explained by the higher energy expenditure and thus higher endogenous acetone production of children as compared to adults. The younger the child the higher the endogenous acetone levels (Paper III, Table 4). Healthy levels of acetone in adults are considered to be about 13 mg/l.[137] However, no information regarding healthy levels in children is available. As a comparison, the average endogenous level of 3 months old children is 16 mg/l (Paper III, Table 4). Thus, there is a possibility that children tolerate a higher body burden of acetone than adults.[138,139] It may also be that they are more sensitive to the effects of ambient exposure due to the elevated endogenous production. Extra caution may therefore be justified when addressing children's risk of adverse effects from the exposure to acetone.

6.2.2 Gender differences

Slight gender differences in the toxicokinetics of toluene, styrene and methyl chloride were observed (Paper IV, Appendix A and B). Thus, the $CSAF_{HK}$ based on metabolized amount (styrene and methyl chloride) were somewhat higher for males than for females. For chronic effects of toluene and for acute effects for toluene and styrene, female workers had slightly higher factors. No gender differences were observed for acetone (Paper III, Tables 6, 7 and 8).

The gender differences may in part be explained by a lower simulated blood flow to the fat compartment(s) per kg body weight in females (Paper IV, Tables 2 and 3). Hence, females may have higher internal doses of lipophilic solvents and a prolonged exposure due to a slower redistribution from fat tissue after exposure has ended.[26] The gender differences found for the two most lipophilic compounds in the MC simulations; toluene (partition coefficient between fat and blood, $P_{FatB} = 54$) and styrene ($P_{FatB} = 49$), are also the most pronounced. Hence, the differences were found to be smaller for methyl chloride ($P_{FatB} = 6$) and in the case of acetone ($P_{FatB} = 0.23$), not detected. However, the differences between genders are small and could therefore also be the effect of scaling differences in the four PBPK models.

6.2.3 Workplace conditions

Fluctuations in exposure level increased the $CSAF_{HK}$ derived for acetone, toluene, styrene and methyl chloride. Conditions of rapid air-exchange increased the factors more than slow air-exchange, especially those based on acute effect markers. Combined effects of fluctuations in exposure level and workload further increased the $CSAF_{HK.}$

The $CSAF_{HK}$ based on chronic markers of effect were all below the default factor of 1.7-2.2 commonly used for workers (section 3.3.1), also when fluctuations in exposure level and workload were combined. In contrast, the factors for toluene, styrene and methyl chloride based on acute effects exceeded the default value up to three times (Figure 13).

The higher factors obtained for acute effects may be explained by a more direct relationship of the acute surrogate dose (maximum concentration in blood) with fluctuations in ambient exposure level. The factors for acetone were not as markedly affected by such fluctuations as the ones for the other three solvents. The difference is probably due to the higher blood solubility of acetone compared to toluene, styrene and methyl chloride. Indeed, the influence of fluctuations in exposure level was especially pronounced for methyl chloride, which has the lowest blood solubility of the four solvents. Overall, the results indicate that fluctuations in exposure level and workload may be important to consider in the process of setting occupational exposure limits or guidelines.

6.2.4 Comparison with previous work

A limited number of studies have previously used probabilistic PBPK modeling to derive assessment factors for intraspecies toxicolinetic variability of inhaled organic solvents.[140-148] The work performed in this thesis is an attempt to include more of the available information. Thus, similar to the study by Pelekis and coworkers,[143] the work in Papers III and IV includes the effect of physical activity on the surrogate doses. In addition, the simulations herein depart from population distributions of model parameters which were calibrated to, and updated by chemical specific toxicokinetic data in a statistical framework.

Two previous studies have investigated the effect of age on toluene and styrene toxicokinetics, respectively.[141,146] Nong and Krishnan reported the child to adult difference ($95^{th}/50^{th}$ percentile) of the internal dose of toluene to be within a factor of 2.[141] Similar results were obtained in this thesis, where a factor of 1.6 was derived to cover 95 percent of children's surrogate doses (Paper IV). For styrene, Valcke and Krishnan arrived at factors of 1.4 in the adult population, as well as for children ($95^{th}/50^{th}$ percentiles).[146] It can be compared to the $CSAF_{HK}$ of 1.2 for adults, and 2.1 for children obtained in this thesis (Paper IV, Appendix A). The higher factor for children obtained herein is most probably due to the higher uptake of solvent vapour during physical activity.

The impact of gender and/or fluctuations in exposure level and workload on the toxicokinetic variability among workers has not previously been examined within the context of assessment factors.

6.3 CONCLUDING REMARKS

A population PBPK framework for the derivation of data-driven assessment factors for intraspecies toxicokinetic differences ($CSAF_{HK}$) was developed, and applied on four organic solvent with different properties (acetone, toluene, styrene and methyl chloride). The $CSAF_{HK}$ which were obtained are well suited to replace the default value commonly deployed for establishing exposure guidelines or limits. The population framework has many advantages. It includes various types of data from already conducted experiments and allows for $CSAF_{HK}$ to be calculated not only for the population as a whole but also for different subpopulations of interest. The framework can be extended to include other chemical substances than the ones presented in this thesis, and also to incorporate additional information on human variability when such becomes available. Thus, the work presented herein may contribute to establish more reliable exposure standards for chemical substances.

The $CSAF_{HK}$ for acetone (excluding endogenous variability), toluene, styrene and methyl chloride derived for the general population were at most 2.4, and thus below the commonly used default factor of 3.16, also for possibly sensitive sub-populations. Fluctuations in exposure level and workload at the workplace have not previously been examined within the context of assessment factors. In this thesis they were shown to increase the $CSAF_{HK}$ for acetone, toluene, styrene and methyl chloride. The factors reflecting acute effects of solvent exposure were up too nearly three times higher than the default value typically used for inter-worker variability (1.7-2.2). Thus, fluctuations in exposure level and workload may be important factors to consider when occupational exposure limits or guidelines are derived. Given the diverse properties of these four volatiles, the results can probably be applied on inhalation exposure to organic solvents in general. Higher factors may however be obtained for substances for which the toxic effect is mediated by an active metabolite, and a majority of the population either lacks or has low capacity for this pathway.

To further develop the methodology presented in this thesis, additional data on physiological parameters are required, in particular for adult females and children. In order to improve the estimates of toxicokinetic population variability of inhaled organic solvents, it may be especially important to increase knowledge on the parameters determining the physiological changes occurring during physical activity.

During Bayesian calibration of the acetone PBPK model, it was shown that the choice of prior distributions had a strong influence on the results. Thus, the use of Bayesian methods in toxicological risk assessment may need to be further explored when it comes to prior selection, and the impact such may have on the outcome.

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