Year : 2011

Biological monitoring of chemical exposure - Toxicokinetic differences due to age and sex.

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Biological monitoring of chemical exposure – Toxicokinetic differences due to age and sex.

Thèse de doctorat ès sciences de la vie (PhD)

présentée à la

Faculté de biologie et de médecine
de l'Université de Lausanne

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Lausanne 2011

UNIL | Université de Lausanne
Faculté de biologie
et de médecine

Ecole Doctorale

Doctorat ès sciences de la vie

Imprimatur

Vu le rapport présenté par le jury d'examen, composé de

Président	Monsieur Prof. Vincent **Barras**
Directeur de thèse	Madame Prof. Brigitta **Danuser**
Experts	Monsieur Prof. Thierry **Buclin**
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le Conseil de Faculté autorise l'impression de la thèse de

Madame Catherine Tomicic-Schroeder

Chimiste diplômée de l'EPFL

intitulée

Biological monitoring of chemical exposure - Toxicokinetic differences due to age and sex

Lausanne, le 13 avril 2011

pour Le Doyen
de la Faculté de Biologie et de Médecine

Prof. Vincent Barras

To Pierre-Olivier Droz

~

Fir eis léif Huesemamm

Acknowledgements

A PhD project would, in my opinion, not be achievable without the precious support of the surrounding people. There is above all one person that I want to thank profoundly, the late Dr Pierre-Olivier Droz. I had the chance to discover the field of occupational health through my diploma work, which was at that time under the supervision of Dr Pierre-Olivier Droz. This project was one of the most interesting one I had to deal with during my whole studies, which gave me the motivation to continue with a PhD project.

The beginning was quite difficult because of lack of funding, but thanks to the support of Dr Pierre-Olivier Droz, and Dr Marc Kremer from the Health Ministry in Luxemburg, grants were finally acquired. My thanks go thus to the institutions that provided financial support for the present research work: the National Research Fund (FNR) in Luxembourg, the French Agency of Environmental and Occupational Health Safety (Anses) and the Swiss Federal Office of Public Health (FOPH).

Moreover, through Dr Pierre-Olivier Droz's contacts, I had the great opportunity to pass a month with an impressive research group from the Karolinska Institutet in Stockholm, Sweden. It was an amazing experience to work with the group of Professor Gunnar Johanson. I want to thank them deeply for their warm welcome and for the insightful discussions. I thank above all Anna-Karin Mörk, who took care of me and who transmitted me a lot about toxicokinetic modelling.

Thanks to the IST, I had the chance to follow a Master of Advanced Studies in occupational health. Without Dr Pierre-Olivier Droz, I would not have had the chance to carry out my diploma work in the frame of the MAS in Benin, under the supervision of Professor Benjamin Fayomi. Once more, the welcome was great and the exchange with the students of Professor Benjamin Fayomi was rewarding.

I also thank Dr David Vernez, who took over the supervision of this PhD project. He was always available, also during the time I left the institute for a new job.

I then want to thank Professor Brigitta Danuser. When there was a decision to be taken, she was the one who knew how to manage the situation, allowing the project to go on.

My thanks go also to Dr Michèle Berode, for her support and her availability. Her group at the laboratory also gave me always the needed support. Among others many thanks go to Ms Patricia Stephan, Ms Monique Strebel, Ms Julia Bersier, Ms Mégane Volet and Ms Nadège Gavillet for their skillful technical work. And a special thank goes to Ms Christine Arnoux who transmitted me a lot, about analytical methods as about the exposure chamber.

The physicians also gave me a big support during the whole experimental part of the project. I want to thank Dr Marcel-André Boillat, Dr Sophie Maria Praz-Christinaz, Dr Gilles Bieler, Dr Frédéric Regamey, Dr Bastien Chiarini, Dr Roland Yerly and Dr Bernd Zeilfelder for their medical assistance.

I also express my thanks to the group of Professor Vincent Castella, especially Fabienne Leyvraz, who contributed to the project by carrying out the participants' genotype.

I also want to thank Professor Thierry Buclin, who contributed at the very beginning to the development of the PhD project, and who stayed available for any of my questions during the work.

Other persons had an important role during the project: my lovely office colleagues. First of all I want to thank M. Horacio Herrera, M. Pierre-Alain Porchet and Ms Nicole Charrière for their general support throughout the whole project.

I would also like to thank Yann Randin and Jean-Noël Lepdor for their technical assistance and for keeping alive the old gas chromatograph.

A special thank goes to Aurélie Berthet, Jérôme Lavoué, Antoine Milon, Marie-Louise Vogt, Magdalena Sanchez-Sandovàl, Thomas Rinsoz and Kaspar Schmid for their support, the great coffee breaks and their friendship.

I want to address my deepest thanks to my new work colleagues from the Federal Office of Public Health, especially Dr Pierre Crettaz, who supported me throughout the new job and the finalization of the PhD.

Outside work, there is another group of persons whom I want to express my warmest thanks: my flamenco professor Sylvia Perujo and the whole dancing group.

I want now thank another very important person, without whom I would not be there where I am now: Professor Dolphe Kutter, close friend of the family, who contributed a lot to the good progress of my studies.

Thank you to my whole family! Although they are spread over the whole world, they gave me regular support. But a special thank goes to my parents, my grandparents, my godfather, my brothers and sisters.

My friends also played an important role throughout the project. First special thanks to my best friend Françoise Probst. I also thank my closest friends Winnie Man, Nadia Kashyap, Sylvie Kersch, Serge Thill (thanks for proofreading!), Guy Bollendorf, Béatrice Lecroq and Davor Trumbic, Cira Hamo, Marie Cornut, Raphaël Nguyen, Stéphane Suarez, Fanny Masson, Pascal Purro and Yves Moret, Fabien Python and Stéphanie Romanens.

Finally, I want to express my profound thanks to my love Claudio, who cared about cooking delicious dishes during the last months of finalization. And lastly special thanks to my cuddly cats.

Abstract

Human biomonitoring is a widely used method in the assessment of occupational exposure to chemical substances and recommended biological limits are published periodically for interpretation and decision-making. However, it is increasingly recognized that a large variability is associated with biological monitoring, making interpretation less efficient than assumed. In order to improve the applicability of biological monitoring, specific factors responsible for this variability should be identified and their contribution quantified. Among these factors, age and sex are easily identifiable, and present knowledge about pharmaceutical chemicals suggests that they play an important role on the toxicokinetics of occupational chemical agents, and therefore on the biological monitoring results.

The aim of the present research project was to assess the influence of age and sex on biological indicators corresponding to organic solvents. This has been done experimentally and by toxicokinetic computer simulation. Another purpose was to explore the effect of selected CYP2E1 polymorphisms on the toxicokinetic profile.

Age differences were identified by numerical simulations using a general toxicokinetic model from a previous study which was applied to 14 chemicals, representing 21 specific biological entities, with, among others, toluene, phenol, lead and mercury. These models were runn with the modified parameters, indicating in some cases important differences due to age. The expected changes are mostly of the order of 10-20 %, but differences up to 50 % were observed in some cases. These differences appear to depend on the chemical and on the biological entity considered.

Sex differences were quantified by controlled human exposures, which were carried out in a 12 m^3 exposure chamber for three organic solvents separately: methyl ethyl ketone, 1-methoxy-2-propanol and 1,1,1-trichloroethane. The human volunteer groups were composed

of ten young men and fifteen young women, the latter subdivided into those with and without hormonal contraceptive. They were exposed during six hours at rest and at half of the threshold limit value. The kinetics of the parent compounds (organic volatiles) and their metabolite(s) were followed in blood, urine and expired air over time. Analyses of the solvent and their metabolites were performed by using headspace gas chromatography, CYP2E1 genotypes by using PCR-based RFLP methods. Experimental data were used to calibrate the toxicokinetic models developed for the three solvents. The results obtained for the different biomarkers of exposure mainly showed an effect on the urinary levels of several biomarkers among women due to the use of hormonal contraceptive, with an increase of about 50 % in the metabolism rate. The results also showed a difference due to the genotype CYP2E1*6, when exposed to methyl ethyl ketone, with a tendency to increase CYP2E1 activity when volunteers were carriers of the mutant allele. Simulations showed that it is possible to use simple toxicokinetic tools in order to predict internal exposure when exposed to organic solvents. Our study suggests that not only physiological differences but also exogenous sex hormones could influence CYP2E1 enzyme activity.

The variability among the urinary biological indicators levels gives evidence of an interindividual susceptibility, an aspect that should have its place in the approaches for setting limits of occupational exposure.

Résumé

La surveillance biologique est de plus en plus utilisée dans l'évaluation d'expositions professionnelles des travailleurs. Il existe des normes biologiques pour plusieurs substances chimiques qui ne prennent pas forcément en compte la variabilité interindividuelle. Lorsque celle-ci est associée aux systèmes biologiques, les décisions sont souvent difficiles à prendre et l'interprétation des résultats n'est pas toujours évidente. Afin d'améliorer l'application de la surveillance biologique, il est essentiel d'avoir de très bonnes connaissances de l'impact de certains paramètres biologiques, comme l'âge et le sexe.

Le but général de cette étude était d'évaluer l'influence de l'âge et du sexe sur les indicateurs biologiques d'expositions correspondants à des solvants organiques, soit de manière expérimentale, soit par la modélisation toxicocinétique. De plus, l'effet de certains génotypes du CYP2E1 sur l'activité enzymatique a été étudié à titre exploratrice.

Les changements toxicocinétiques liés à l'âge ont été évalués par modélisation, à partir d'un modèle toxicocinétique simple d'une étude précédente qui a été appliqué à 14 substances chimiques, représentant 21 déterminants biologiques, avec, entre autres, le toluène, le phénol, le plomb ainsi que le mercure. Ces modèles toxicocinétiques ont été modifiés en prenant en compte l'âge. Après simulation, des différences de l'ordre de 10 à 20 % ont été observées dans la plupart des cas. Néanmoins, pour quelques substances chimiques, des différences jusqu'à 50 % ont pu être mises en évidence. Elles semblent dépendre du produit chimique et de l'indicateur biologique considéré.

Des études expérimentales en cabine d'exposition ont permis d'évaluer l'importance des changements toxicocinétiques dus au sexe pour trois solvants organiques: la méthyléthylcétone, le 1-méthoxy-2-propanol et le 1,1,1-trichloroéthane. Les groupes de

volontaires étaient composés de dix jeunes hommes et de quinze jeunes femmes, dont dix prenant un contraceptif hormonal. Les participants étaient exposés, au repos pendant six heures, à la moitié de la valeur moyenne d'exposition du solvant considéré. Les indicateurs biologiques respectifs ont été suivis dans l'urine, le sang ou l'air expiré. Les analyses des substances mères et des métabolites ont été effectués par la technique dite headspace en chromatographie en phase gazeuse, le génotypage par une technique de PCR/RFLP. Les données expérimentales ont servi à valider les modèles toxicocinétiques développés pour les trois solvants organiques à partir d'un modèle général existant. Des différences dans les niveaux d'indicateurs biologiques ont principalement été observées chez les femmes. Les participantes sous prise de contraceptif hormonal ont montré un taux de métabolisme augmenté de 50 %. Les résultats ont également indiqués que les volontaires porteurs de l'allèle mutant d'un des génotypes étudiés, le CYP2E1*6, auraient une tendance à avoir une activité enzymatique plus élevée. Les simulations obtenues à partir des modèles toxicocinétiques montrent qu'il est possible d'utiliser un modèle général simple afin de prédire l'exposition interne d'une personne lorsque celle-ci est exposée à des solvants organiques. Cette étude suggère que les différences toxicocinétiques observées entre hommes et femmes ne sont pas essentiellement dues aux différences physiologiques. Les hormones sexuelles, et notamment les variétés exogènes, semblent jouer un rôle important dans l'activité du CYP2E1.

La variabilité interindividuelle est par conséquent un aspect qui doit avoir sa place dans l'élaboration de valeurs limites professionnelles.

List of publications

Peer-reviewed articles (published)

Tomicic, C., Droz, P.-O. (2009) Age differences in biological monitoring of chemical exposure: a tentative description using a toxicokinetic model. *International Archives of Occupational and Environmental Health* 82(5), 669-676.

Tomicic, C., Berode, M. (2010) Sensitive headspace gas chromatography analysis of free and conjugated 1-methoxy-2-propanol in urine. *Analytical and Bioanalytical Chemistry* 396(7), 2709-2714.

Tomicic, C., Berode, M., Oppliger, A., Castella, V., Leyvraz, F., Praz-Christinaz, S.M., Danuser, B. (2011) Sex differences in urinary levels of several biological indicators of exposure: a human volunteer study. *Toxicology Letters* 202(3), 218-225.

Peer-reviewed articles (submitted)

Tomicic, C., Vernez, D. Sex differences in urinary levels of several biological indicators of exposure: a simulation study using a general compartmental based toxicokinetic model. Submitted in *Journal of Occupational and Environmental Hygiene*.

Oral communication

Tomicic, C. (2009) Prise en compte de la susceptibilité individuelle dans la population vieillissante. Meeting with experts in setting threshold limit values for chemical agents in the workplace held at the French Agency of Environmental and Occupational Health Safety (Anses - Agence nationale de sécurité sanitaire de l'alimentation, de l'environnement et du travail) in Paris.

Written communications

Tomicic, C., Droz, P.-O., Berode, M., Boillat, M.-A., Buclin, T. (2007) Les différences toxicocinétiques dues à l'âge et ses conséquences pour la surveillance biologique de l'exposition professionnelle. Meeting at the Association for Research in Toxicology (Association pour la Recherche en Toxicologie) held in Paris.

Tomicic, C., Berode, M., Praz-Christinaz, S.M., Danuser, B., Droz, P.-O. (2009) Influence of gender on the levels of biological indicators of exposure. Scientific meeting organized on 15th May 2009 in Luxemburg by the National Health Laboratory and supported by the National Science Foundation of Luxemburg.

Tomicic, C., Droz, P.-O., Berode, M., Praz-Christinaz, S.M., Castella, V. and Danuser, B. (2010) Controlled human exposures to organic solvents: influence of sex on urinary levels of biological indicators. XII International Congress of Toxicology (IUTOX) held from the 19th to the 23rd of July in Barcelona, Spain. Abstract in Toxicology Letters 196S, 39.

Abbreviations

ACGIH	American Conference of Governmental Industrial Hygienists (Cincinnati, Ohio, USA)
BEI	Biological Exposure Indice
CYP2E1	Cytochrome P450 2E1, a member of the cytochrome P450 mixed-function oxidase system
DFG	Deutsche Forschungsgemeinschaft (Bonn, D)
GC	Gas chromatograph
IST	Institute for Work and Health (Lausanne, CH)
MEK	Methyl ethyl ketone
1M2P	1-Methoxy-2-propanol
PCR	Polymerase chain reaction
RFLP	Restriction fragment length polymorphism
SUVA	Schweizerische Unfallversicherungsanstalt - Caisse nationale suisse d'assurance en cas d'accidents - Instituto nazionale svizzero di assicurazione contro gli infortuni (Lucerne, CH)
TCA	Trichloroacetic acid
111TCE	1,1,1-Trichloroethane
TCE	Trichloroethanol
TK	Toxicokinetic
TLV	Threshold Limit Value
WHO	World Health Organisation

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1 Introduction

1.1. Context and motivation

Since a long time, researchers like Alice Hamilton (1869-1970), the first woman appointed to the faculty of Harvard Medical School and a leading expert in the field of occupational health, were interested in pointing out the differences in women's and men's working and living conditions, and how these could differentially affect their health. It is only about the end of our century that sex differences in occupational health began seriously to be taken into account (Messing and Mergler, 2006).

Recent publications clearly underlined that we have still little understanding of the impact that sex and gender may have on exposure to chemicals, and that more research on women is necessary and desirable (Ernstgård et al., 2003; Messing and Stellman, 2006; Arbuckle, 2006; Gochfeld, 2007).

Another concern was underlined by Illmarinen (2001). He presented the main conclusions from European Union statistics on population aging, as for example the fact that workers aged of 50 years and more will constitute the largest proportion (> 30 %) of the work force during the next 25 years.

The Swiss Federal Statistical Office published several scenarios of the population evolution until year 2050 (SFSO, 2010). After the average scenario, the proportion of children and teenagers (0-19 years) will stay more or less stable, with 20.8 % in 2010 and 18.4 % in 2060, whereas the proportion of old people (> 64 years) will increase from 17.1 % to 28.3 % during the same period. As a matter of fact, the peak of the population pyramid will become gradually broader in the next fifty years while its basis stays unchanged. The active population will get old in an unquestionable way during this period.

The understanding of the influence of age and sex is determinant in the context of our new economic and work situation. Workers are getting older and older, and as a consequence their medication is also increasing (WHO, 2002). Moreover workers are not anymore specifically male.

Biological monitoring is frequently used in exposure assessment and in surveillance of workers over time. Biological exposure limits are known for many chemicals and are published in several countries (ACGIH, 2010; DFG, 2009) and in Switzerland (SUVA, 2009). However variability associated with biological systems often precludes a sound interpretation and decision making. To improve applicability of biological monitoring it is essential to have more information, and notably to understand the effect of specific modifying factors, such as age and sex. With this information, a prior distribution of expected results could be defined and serve a better decision making process. This project should contribute to an improvement of biological monitoring procedures for the specific chemicals studied, but also for other chemicals, using toxicokinetic modelling techniques.

1.2. Biological monitoring of occupational exposure

Biological monitoring, which relies on measurements of the chemical or its metabolites in biological fluids, is integrated since a long time as a fundamental exposure assessment tool in the field of occupational health.

The first paper on biomonitoring appeared in 1927 and reported the usefulness of the determination of urinary lead when diagnosing lead-induced industrial disease (Aitio, 2006).

Besides metals, the organic solvents represented another important family of substances on industrial scale. Their production and use increased mainly in the latter half of the 19th century due to the coal-tar industry. They can be mainly classified as aliphatic hydrocarbons,

cyclic hydrocarbons, aromatic hydrocarbons, halogenated hydrocarbons, ketones, amines, esters, alcohols, aldehydes, ethers and glycol ethers. During the 1980's, a major concern for occupational hygienists and physicians was to assess the risk of occupational exposure to volatile organic compounds (Droz et al., 1983) as their neurotoxicity represented one of the most important emerging issues in the field of occupational health (Baker 1988).

Due to their chemical properties, organic solvents are still commonly used worldwide in different industrial sectors as degreasing and diluting agents.

A key component in protecting workers from chemical agents is the concept of biological exposure indices (BEIs), which are reference values used as guidelines for the evaluation of potential health hazards related to a substance (Morgan, 1997). The large industrial use of metals and organic solvents over the last decades and in parallel the scientific studies related to them contributed to the establishment of the first recommended biological exposure limit values. Most BEIs are based on a direct correlation with the threshold limit values (TLVs). The latter refer to airborne concentrations of chemical substances and represent conditions under which it is believed that nearly all workers may be repeatedly exposed, day after day, over a working lifetime, without adverse health effects (ACGIH, 2010).

Biological monitoring results are in many cases interpreted on an individual basis, despite their quite often large variations for the same individual over time and between individuals. These variations in biological levels are usually not associated with corresponding exposure changes, nor with health effects, as biomarkers are very often not the toxicologically active species, at least for solvents. Biomarkers are thus in most cases used as indirect indicators of exposure, assuming a fixed relationship between them for a given chemical. Any factor affecting this relationship will thus introduce some uncertainty reducing decision making possibilities.

Recently more and more attention has been devoted to variability associated with biological monitoring results (Pierrehumbert et al., 2002; Tardif et al., 2002; Truchon et al., 2006; Berthet et al., 2010; Thomson et al., 2010). This aspect of biological monitoring was often not considered and results were used without paying much attention to the associated uncertainty. Several studies in volunteers and with toxicokinetic models have now shown that biological results follow approximate lognormal distributions, characterized by geometric standard deviations ranging from about 1.3 to 2.0 (Droz, 1992), equivalent to a coefficient of variation between 30 and 100 %. This large biological variability was already obvious to drug therapists (Meibohm, 2002; Schwartz, 2003), and adaptation of dosage is quite current practice, at least for drugs having narrow therapeutic ranges.

Among others, age and sex are easily identifiable determinants which could play an important contribution to biological variability. There is still little understanding of the influence of these determinants on exposure to and measurement of occupational chemicals. Several authors recommended recently that more toxicokinetic studies should be undertaken to better characterize the influence of sex and age (Arbuckle, 2006; Messing, 2006; Gochfeld, 2007).

In a recent review, Truchon et al. (2004) indicated that the question of the influence of age on industrial chemical toxicokinetics has only received little attention until now. They recommended that this aspect should be further studied, notably by toxicokinetic modelling, to better understand its contribution to biological variability. Truchon et al. (2004), referring to Canadian statistics, mentioned that the proportion of aged workers will represent 39 % of the active population in 2016, compared to 29 % in 1996.

Few studies exist about the possible evaluation of sex differences in the toxicokinetics of occupational toxicants in humans. Ernstgård et al. (2003a, 2003b) exposed male and female volunteers to m-xylene and 2-propanol under controlled conditions. They found toxicokinetic

modifications for both chemicals, which they attributed mainly to anatomical differences between sexes.

Biological variability has been taken into account very early in risk assessment procedures, probably because the extrapolation between animal species and from high to low doses forced the discussion of uncertainty (Dorne, 2005). In that case factors were used to describe uncertainty, leading to the establishment of default safety factors (WHO, 1987). Recently, attempts have been made to improve or reduce these factors by using various modelling techniques for extrapolation (WHO, 2001).

Several authors started to describe, using various modelling tools, the contribution of age and sex to biological variability (Hattis et al., 2003; Clewell et al., 2002). For example, Clewell et al. (2004) developed a toxicokinetic model in order to determine the tissue concentration as a function of time of some xenobiotics by including age and sex as important parameters, representing an initial attempt to provide a predictive toxicokinetic framework.

1.3. Toxicokinetic modifications due to age and sex

Toxicokinetic behaviour of chemicals in the body determines biological levels, and thus biological indicator results. It is usually described as four processes: absorption, distribution, metabolism and elimination. Biological variability results from changes in any of these four processes.

1.3.1. Absorption

In occupational settings, absorption can occur by inhalation, by ingestion or by skin contact. In the present project, only inhalation will be considered.

Upon aging, lungs become stiffer, muscle strength and endurance diminish, and the chest wall becomes more rigid. Total lung capacity remains constant but vital capacity decreases and

residual volume increases. The alveolar surface area decreases by up to 20 percent. Alveoli tend to collapse sooner on expiration. There is a decrease in the activity and number of cilia and an increase in mucus production. All these changes can contribute to reduce the transfer of a substance through the pulmonary membrane. When the inhaled product is gaseous, the most probable impact of aging on the pulmonary absorption of the substance is a decrease in absorption because of the corresponding modifications occurring in the lungs (Wahba, 1983; Tockman, 1990). For contaminants present as aerosols, (Elder et al., 2000), there could be a decrease in the absorption of the smallest particles. One explanation is that with age, there is a possible accumulation of particles in the lungs.

For sex differences in absorption by inhalation, only limited data are available (Schwartz, 2003). Overall there are few demonstrated differences in absorption from lung between men and women. Ernstgård et al. (2003a,b) carried out a study with volunteers in order to evaluate possible sex differences in the inhalation toxicokinetics of a polar (2-propanol) and a nonpolar solvent (m-xylene). A limitation of their study was a low number of participants and a low exposure level. Nevertheless, the results indicated slight differences between men and women. For m-xylene, it seems that body size and body composition were more important determinants than sex. For 2-propanol, body build could not explain the difference between women and men regarding 2-propanol in expired air and acetone, one of its metabolites, in saliva.

1.3.2. Distribution

The principal modifications in distribution are due to changes in body composition in the elder human. There is a progressive reduction in total body water (about 15 %) and in lean body mass, resulting in a relative increase in body fat (about 30 %) (Truchon et al., 2004). These changes have an influence on the volume of distribution of the substance. Hence

hydrophilic substances tend to have smaller volumes of distribution resulting in higher plasma concentration, while for lipophilic substances the volume of distribution increases with age and the plasma concentration decreases (Turnheim, 2003).

The distribution of a chemical agent can also be altered by modifications in protein binding. A decrease of about 15 to 20 % is often observed with age in the plasma concentration of albumin which binds to many industrial solvent or metabolites (Lichtman, 1995). One can therefore expect an increase in free or unbound chemical concentration.

Female physiological differences such as lower average body weight, higher body fat composition, smaller plasma volume and lower average organ blood flow can modify the rate and extent of distribution of the chemical in women (Gandhi et al., 2004). Lipophilic substances will tend to have a greater distribution volume in women, hydrophilic ones will show the opposite (Schwartz, 2003).

The binding to proteins in the plasma is also influenced by sex hormones, which can lead to toxicokinetic differences between sexes (Gandhi et al., 2004). Albumin is a major plasma protein responsible for reversible drug binding, but not really affected by sex (Verbeeck et al., 1984). Schwartz (2003) wrote about an early interest in reports of sex-related differences in α_1-acid glycoprotein concentrations. The protein binding seems to be greater in men than in women.

1.3.3. Metabolism

The major metabolic organ in the body is the liver. The age-related changes of the liver are a decrease of the hepatic mass and of hepatic blood flow (Sellers, 1989; Vestal, 1997). The hepatic mass can be decreased by about 20 to 50 %, leading to a reduction of the metabolic capacity (George et al., 1995; Vestal, 1997). Since age 25, the total hepatic blood flow diminishes from 0.5 % to 1.5 % every year. For a person of 65 years old, this causes a

reduction in hepatic blood flow of 35-40 % (Vestal, 1997; LeCouteur and McLean, 1998). Hence this could reduce metabolic elimination of a chemical that shows a flow-limited behavior.

Cytochrome P450 dependent mono-oxygenases are the most prominent enzymes involved in solvent metabolism. Isoenzymes belonging to the first four gene families (CYP 1-4) are involved in the metabolism of xenobiotics (Löf et al., 1998). The metabolism of many solvents includes oxidation by the CYP2E1 (Nakajima et al., 1997). This specific isoform, probably among others, is known to have an activity increasing with age, to exhibit sex differences and to show a polymorphism (Bebia et al., 2004). It is well known that the overall P450 activity is also changing with age, more specifically for some P450 isoforms, such as the CYP3A family.

Studies showed that the decrease in metabolism with increasing age seems to be mainly related to changes in the activity of liver enzymes (Geller and Zenick, 2005), while others mentioned that it could come from the decline in liver volume and blood flow (Schmucker, 2001). In both cases the result can be a slowed detoxification of chemical compounds.

Anantharaju et al. (2002) mentioned that the majority of the drug interactions in the elderly is related to the hepatic metabolism by multiple enzyme systems including cytochrome P450, which is supported by a study done by Sotaniemi et al. (1997). The conclusion is the following: “This study shows a reduction of *in vitro* and *in vivo* drug metabolism with age in humans. The data suggest that at least three age groups—young, middle-aged, and elderly—should be included in the evaluation of the pharmacokinetics of a new drug. Cytochrome P450 content in subjects from 20 to 29 years of age was 7.2 ± 2.6 nmol · gm^{-1}, increased during the fourth decade (+ 7.2%, p = NS), declined after 40 years (−16%, $p < 0.01$) to a level that remained unaltered up to 69 years, and declined further after 70 years (−32%, $p < 0.001$).”

LeCouteur and McLean (1998) proposed an alternate mechanism based on oxygen delivery (phase I enzymes are directly dependent on oxygen supply as a substrate) in order to try to resolve this paradox of phase I drug metabolism presented by Schmucker (2001). McLean and Le Couteur (2004) concluded that "hepatic clearance of flow-limited drugs is reduced secondary to age-related reduction in hepatic blood flow, and drug clearance may also be influenced by age-related changes in hepatic sinusoidal endothelium."

A large body of literature comparing clearance of hepatically metabolized substances in men and women exists regarding the differences in metabolism due to sex (Schwartz, 2003). The large interindividual variability in amount and activity of the individual enzymes makes it difficult to demonstrate sex-dependent differences in metabolism (Anderson, 2005). According to Schwartz (2003), most metabolic routes show a higher or similar clearance in men compared to women. But differences have been identified in activities for CYP1A2, CYP3A4, CYP2D6, and CYP2E1. Those enzymatic activities are usually higher in males than in females (Tanaka, 1999; Clewell et al., 2002; Parkinson et al., 2004).

1.3.4. Excretion

The most important pharmacokinetic change in the elderly is the reduction in renal elimination (Turnheim, 2003). The age-related changes for the kidneys are a decrease in the renal mass and renal blood flow. Between 40 and 90 years, the decrease of the renal mass is about 30 % (Yuen, 1990; Evers et al., 1994; Gleason, 1996). Glomerular filtration decreases about 1 ml/min per year after 40 years (Evers et al., 1994). The renal blood flow diminishes about 1 % per year after the age of 50 (Yuen, 1990; Gleason, 1996; Vestal, 1997). There are also other renal functions like the renal clearance that decrease with the elderly (Evers et al., 1994). The urinary excretion of creatinine is also affected by age. At 65 years, there is a decrease of about 15 to 25 % comparatively to someone of 20 years old. This change is

explained by a decrease of renal mass and glomerular filtration (Alessio et al., 1985; Boeniger et al., 1993). These modifications lead to a reduction of the elimination of chemicals and their corresponding metabolites (Evers et al., 1994; Vestal, 1997).

Sex-related differences in glomerular filtration, tubular secretion and tubular reabsorption have been pointed out (Schwartz, 2003). Glomerular filtration rate is 10 % lower in females than in males after correction for body size (Gross et al., 1992). The effect of sex on renal secretion and renal reabsorption is less clear (Schwartz, 2003). Creatinine clearance rates are generally higher in males than in females and lower during the first week of menses than later in the cycle (Arbuckle, 2006).

1.4. Organic solvents

Due to their chemical properties, organic solvents are a family of chemical substances that are commonly used worldwide in different industrial sectors as degreasing and diluting agents or in consumer products as detergents or emulsifiers. The experimental part of the present study focused on chemical exposures to organic solvents and in order to be able to quantify toxicokinetic differences due to sex, three solvents have been selected based on their wide practical use, their relatively low toxicity, and their metabolic and physiological properties.

Methyl ethyl ketone (MEK), with a TLV of 200 ppm (Suva, 2009), is water soluble and highly metabolized. MEK is metabolized to 2,3-butanediol, 2-butanol and 3-hydroxy-2-butanone (major metabolite), all excreted in urine. A low fraction of unchanged solvent is excreted in the exhaled air and in urine. The urinary concentration of MEK at the end of the shift has been recommended as the most appropriate biological exposure indicator.

MEK is used as a solvent in the application of protective coatings (varnishes) and adhesives (glues and cements). It is also commonly used in paint removers, cleaning fluids, acrylic

coatings, pharmaceutical production, and colorless synthetic resins, and as a printing catalyst and carrier. Occupational exposure can occur mainly by inhalation and by dermal uptake.

The main effects during acute exposure have been described to be irritation symptoms (eye, nose, throat) (INRS, 2009). These are general moderate and can begin at half of the TLV. Recent studies showed that exposure to 200 ppm during 4 hours had no significant effect in a variety of behavioural and psychological tests. Symptoms as headaches or gastrointestinal upset, or even central nervous system disorders, can appear at higher exposure concentrations. Case reports of encephalopathies and peripheral neuropathies have been described during chronic exposures to MEK but in general, exposure occurred to a mixture of solvents. Chronic dermal contact can result in dermatoses. Industrial exposure to moderate levels of MEK is widespread but these levels can be much higher due to inadequate ventilation systems.

1-Methoxy-2-propanol (1M2P), with a TLV of 100 ppm (Suva, 2009), is also water soluble and highly metabolized. 1M2P belongs to the family of the propylene glycol ethers (PGE) which exists under the form of two isomers, alpha-isomer and beta-isomer. The latter one, considered as an impurity in commercial use, is first transformed as all primary alcohols into 2-alkoxy acetaldehydes by alcohol deshydrogenases, and then into alkoxyacetic acids by aldehyde deshydrogenases, a toxic metabolite. Alpha-PGE are primarily metabolized to propylene glycol, carbon dioxide and glucuronide as well as sulfate conjugates of the parent compound. The urinary 1M2P concentration at the end of the shift corresponds as the appropriate biomarker.

1M2P is used in a wide variety of industrial and commercial products, primarily water-based paints, varnishes and inks.

In the case of acute exposure above 100 ppm, main effects are similar to those observed when exposed to MEK, that means irritation symptoms in the area of the nose, the eyes and the

throat (INRS, 2010). Neurological effects are observed for concentrations of 1M2P above 1000 ppm. There is no data in literature about toxic effects in humans due to chronic exposure to 1M2P.

Occupational exposure can occur mainly by inhalation and dermal absorption. A field study (Devanthéry et al., 2000) showed that workers in an ink factory can be exposed to a third or half of the TLV, indicating that air levels of 1M2P in industry can be important.

1,1,1-Trichloroethane (111TCE), with a TLV of 200 ppm (Suva, 2009), is liposoluble and slowly metabolized. 111TCE is metabolised to trichloroethanol (TCE) and trichloroacetic acid (TCA), both excreted in urine. Other minor metabolites (carbon dioxide, acetylene) are excreted in the exhaled air. The corresponding biological exposure indices are the urinary concentration of both metabolites and the blood concentration of the parent compound. The 111TCE concentration in the exhaled air can also be considered as a biomarker of exposure.

111TCE has been largely used particularly as degreasing agent or as a solvent for inks, paints, adhesives and other coatings. The Montreal Protocol targeted 111TCE as one of those compounds responsible for ozone depletion and banned its use beginning in 1996. Thus, its manufacture and use has been phased out throughout most of the world.

111TCE was once used for anesthesia, en effect that could be reached by inhalation of 10 000 to 26 000 ppm (INRS, 2007). Inhaled 111TCE can thus act as a central nervous system depressant and can cause effects similar to those of intoxication, including dizziness, confusion, and in sufficiently high concentrations, cardiac disorder (ventricular fibrillation), unconsciousness and death. Chronic exposure to 11TCE vapors can lead to skin, eyes and respiratory irritations.

The three solvents are mainly absorbed by inhalation and metabolized via the cytochrome P450 mixed-function oxidase system, with the cytochrome CYP2E1 as the main enzyme involved in the metabolism of organic solvents. They are well known and studied, both in volunteers and in workers, but there is no data available on the effect of age and sex on their toxicokinetics in humans.

1.5. Gaps in knowledge

It is presently very difficult to understand quantitatively the variability associated with biological monitoring results. Although we can establish a list of contributing factors, as shown in a framework for age differences by Geller and Zenick (2005) and a framework for sex differences by Gochfeld (2007), their relative importance is unknown for different chemicals of occupational interest. It is therefore presently impossible to use such information to reduce uncertainty due to biological variability and make safer quantitative decisions when using biological monitoring of exposure.

There is only very limited information about changes in biological indicators of exposure with age and sex. When available it is restricted to a few chemicals and to some specific situations only. However, changes in physiological parameters, and even metabolic functions, with age and sex are rather well known. The same is valid when looking at pharmaceutical drugs for which we have a deeper experience. The general goal of the present project is therefore to integrate this available information (namely physiological, metabolic and pharmaceutical) in a quantitative framework in order to be able to understand the effect of age and sex on biological monitoring of occupational exposure.

2 Objectives

The main objective of the present research project was to assess the influence of age and sex on biological levels of some important chemicals and their metabolites in the human body. Compartmental toxicokinetic models developed in previous studies for a series of metallic and organic compounds were used to describe the differences due to age. The approaches applied to study the influence of sex on the chemical disposition of three specific organic solvents (MEK, 1M2P, 111TCE) were human volunteer exposures under controlled conditions in an experimental exposure chamber and toxicokinetic modelling using compartmental models. Furthermore another purpose was to explore the influence of toxicogenetic polymorphism (CYP2E1) on urinary levels of the studied biomarkers.

Results will indicate if the variability due to age and sex is important and if it depends on the chemical. The information obtained, the models developed and the understanding reached will be of great use in interpreting biological monitoring data in occupational exposure assessment. The use of toxicokinetic models will allow a mechanistic description and the extrapolation of data gathered on a few chemicals to other substances of industrial interest. The aim was not to adapt exposure limits for the aged active population and for the male-female differences but it was to better understand the contribution of age and sex to biological variability, in order to take it into account when assessing exposure of individuals.

3 Results

Paper I: Several work in toxicokinetic modelling has been done during the first year of the PhD. Specific factors responsible for interindividual variability should be identified and their contribution quantified in order to improve the usefulness of biological monitoring. Among others, age is an easily identifiable determinant which could have an important impact on biological variability. A compartmental toxicokinetic (TK) model developed in previous studies for a series of metallic and organic compounds was applied to the description of age differences. Young male physiological and metabolic parameters, based on reference man information, were taken from preceding studies and were modified in order to take into account age based on available information about age differences. Numerical simulations using the kinetic model with the modified parameters indicate in some cases important differences due to age. The expected changes are mostly of the order of 10-20 %, but differences up to 50 % were observed in some cases. These differences appear to depend on the chemical and on the biological entity considered. This work has been published in the form of a short communication in *International Archives of Occupational and Environmental Health* 2009, 82(5): 669-676.

Paper II: The analytical work of the project represented an important part, leading to the improvement of one of the existing methods at the institute for the determination of urinary 1M2P. The existing method for the determination of urinary 1M2P included a solid-phase extraction and derivatization before analysis by gas chromatography coupled to a flame ionization detector (Devanthéry et al., 2000). First essays had been done formerly in the frame of a planned study on mouse 1M2P exposure (unpublished results), by modifying the existing method and then by replacing it because it could not achieve enough sensitivity with

microvolumes of biological fluids. Thus, there was an obvious need to develop a sensitive, reliable, alternative method.

Moreover, glycol ethers still continue to be a workplace hazard due to their important use on industrial scale. Currently, chronic occupational exposures to low levels of xenobiotics are increasingly relevant. Thus, sensitive analytical methods for biomarkers of exposure are of interest in the field of occupational exposure assessment.

For 1M2P, with a vapor pressure of 12 mmHg (1.60kPa), the headspace technique was a suitable option to extract this alcohol from biological fluid as applied to different organic volatiles. The determination of urinary 1M2P by the headspace technique was proposed, as it consisted in a highly sensitive and a particularly simple analytical method, useful in the assessment of occupational exposure to 1M2P. Furthermore, the analytical procedure could easily be adapted for other substances of the glycol ethers family if their physico-chemical properties allow it.

In comparison to the former existing analytical method, several aspects have been improved. The influence of the salt addition on the partition coefficients in gas-water systems with the enrichment of compounds of interest in the “head space” phase is a crucial factor to improve the sensitivity of analysis. The limit of detection obtained under the described conditions was much lower (10 fold better). With a limit of detection of 0.1 mg/l, the GC headspace technique allows to determine the target substance in small volumes of biological fluids and/or in low occupational exposures situations. This work has been published under the form of a technical note in *Analytical and Bioanalytical Chemistry* 2010, 396(7): 2709-2714.

Paper III: The main part of this project concerned the experimental study with human volunteers. The aim was to quantify the variability on biological indicators between men and women for three well known solvents: MEK, 1M2P and 111TCE. These three volatile chemicals have been studied both in volunteers and in workers but there is no data available on the effect of sex on their toxicokinetics in humans. Another purpose was to explore the effect of selected CYP2E1 polymorphisms on the toxicokinetic profile.

At the beginning of the project, four different groups of volunteers had been established (young women and young men of 20-25 years, aged women and aged men of 55-65 years) and four chemicals had been chosen for the experimental part of the present study. First human exposures began in December 2007, with six young male volunteers. After these exposure sessions, the next step was to include aged volunteers where major problems have been encountered. During several months, the volunteer research was concentrated on this age category (CHUV, "Université populaire" and "Université du 3ème âge" in Lausanne, announcement in journals and in commercial centers, word-of-mouth advertising). As the experimental protocol was rather restrictive, it was difficult to find any potential aged volunteer as it is a reality that this class of the society often does not have enough time to participate in such a study or is under medication which was contrary to the severe inclusion criteria. The thesis committee proposed at the intermediate evaluation in October 2008 to concentrate the volunteer research especially on young men and women and if necessary to reduce the number of solvents from four to three. Human exposures continued from January 2009 until June 2009. About 40 exposure sessions had been performed in total, with fifteen young women and ten young men.

Controlled human exposures were carried out in a 12 m^3 exposure chamber for each solvent separately, during six hours at rest and at half of the threshold limit value.

Results mainly showed an effect on the urinary levels of several biomarkers among women due to the use of hormonal contraceptive, with an increase of about 50 % in the metabolism rate. Moreover a statistically significant difference due to the genotype CYP2E1*6 with a tendency to increase CYP2E1 activity was observed when volunteers were carriers of the mutant allele.

Our study suggests that exogenous sex hormones could influence CYP2E1 enzyme activity. For same exposure conditions, biological exposure indices can differ between men and women, which can lead to a misinterpretation of biomonitoring results. This aspect should have its place in the approaches for setting limits of occupational exposure. These results have been accepted for publication in *Toxicology Letters* (doi:10.1016/j.toxlet.2011.01.032).

Figure 1 Exposure chamber at the Institute for Work and Health in Lausanne, Switzerland

Paper IV: Pierrehumbert et al. (2002) have developed a general compartmental model in order to quantify the effect of human variability on a wide range of biological exposure indices (BEIs). By applying it to four chemicals (toluene, phenol, lead and mercury), they showed its potential to be used for further substances. We actually underlined this potential of the model by adapting it to three other compounds which are MEK, 1M2P and 111TCE, the three organic solvents chosen for the human volunteer study (see Paper III).

The first step was to calibrate the toxicokinetic models with the experimental data, thus simulation conditions corresponded to the exposure conditions, that means six hours at rest to the half of the threshold limit value. In a second step, simulations were done within working conditions, which means exposure was considered to last 8 h per day, 5 days per week. Physical activity was set at 50 W for 12 h per day, and at rest for the resting 12 hours. The exposure concentration corresponded to the threshold limit value. The simulated urinary levels of the biomarkers were compared to their corresponding biological exposure indices.

Our results showed that it was possible to use an existing generic toxicokinetic model for other compounds by adapting it to every studied organic solvent. In fact, most of the simulation results showed good agreement with the experimental data obtained.

Thus, a general and simple toxicokinetic model, adapted for three well known organic solvents, allowed us to show that metabolic parameters can have an important impact on the urinary levels of the corresponding biomarkers. Indeed, experimental data pointed out that for same exposure conditions, men and women can show a difference of 50 % among the urinary biological indicators levels. Moreover, when running the models by simulating industrial working conditions, these differences could even be more pronounced. Anyway these observations give evidence of an interindividual susceptibility, an aspect that should have its place in the approaches for setting limits of occupational exposure.

3.1. Paper I

Age differences in biological monitoring of chemical exposure. A tentative description using a toxicokinetic model.

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Abstract

Specific factors responsible for interindividual variability should be identified and their contribution quantified in order to improve the usefulness of biological monitoring. Among others, age is an easily identifiable determinant which could play an important impact on biological variability. A compartmental toxicokinetic (TK) model developed in previous studies for a series of metallic and organic compounds was applied to the description of age differences. Young male physiological and metabolic parameters, based on reference man information, were taken from preceding studies and were modified in order to take into account age based on available information about age differences. Numerical simulations using the kinetic model with the modified parameters indicate in some cases important differences due to age. The expected changes are mostly of the order of 10-20 %, but differences up to 50 % were observed in some cases. These differences appear to depend on the chemical and on the biological entity considered. Further work should be done to improve our estimates of these parameters, by considering for example uncertainty and variability in these parameters.

Keywords age · toxicokinetic · modeling · biological indicators

Introduction

Biological monitoring is a method widely used by occupational physicians to estimate chemical exposure. However it is more and more often recognized that a large variability is associated with biological monitoring, making interpretation less efficient than foreseen (Truchon et al. 2006).

Toxicokinetic behaviour of chemicals in the body determines biological levels, and thus biological indicator results. It is usually described as four processes: absorption, distribution, metabolism and elimination. Biological variability comes from changes in these four processes.

In a recent review, Truchon et al. (2004) indicate that the question of the influence of age on industrial chemical toxicokinetics has received little attention. They recommend that this aspect should be further studied, notably by toxicokinetic modeling, to better understand its contribution to biological variability. A few authors have started to describe, using various modeling tools, the contribution of age to biological variability. Some authors have described differences between children and adults (Kreuzer at al. 1997, O'Flaherty 1998, Hattis et al. 2003) and others have also taken in account the elderly (Jeandel et al. 1992, Clewell et al. 2002, Clewell et al. 2004). Hattis et al. (2003) drew on individual data for pharmacokinetic and anthropometric parameters to help define distributions that will be helpful for population distribution modeling of pharmacokinetic differences between children of various ages and adults. Clewell et al. (2004) presented an initial attempt to provide a predictive pharmacokinetic framework in order to evaluate the potential impact of age- and gender-specific differences on risk from chemical exposure. The validation of these chemical-specific model predictions has been made with only young adult data.

It is presently still difficult to understand quantitatively the variability due to age associated with biological monitoring results. Although it is possible to establish a list of contributing

factors, as shown in a framework for age differences by Geller and Zenick (2005), their relative importance is unknown for different chemicals of occupational interest. It is therefore presently unfeasible to use such information to interpret biological variability and make safer quantitative decisions when using biological monitoring of exposure.

In preceeding studies, Truchon et al. (2003) have developed toxicokinetic tools to describe the influence of specific factors on biological levels, such as body build, physical workload, metabolic and excretion functions (Pierrehumbert et al. 2002; Truchon et al. 2006) but not age. One of these tools, namely a simple toxicokinetic model, was applied in the present study to better understand the influence of age on biological exposure indicators. The report in question represents a tentative quantitative estimation of the effect of age on biological levels of several industrial chemicals and their metabolites. It is then applied to specific biological monitoring reference values.

Methods

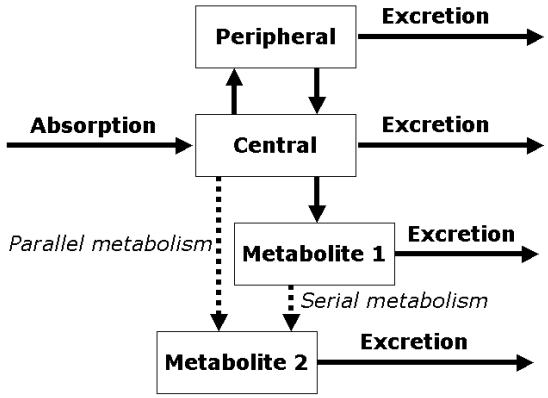
Compartmental toxicokinetic model

A compartmental TK model developed in previous studies (Pierrehumbert et al. 2002) was applied to the description of age differences. The TK model (see Figure 1 taken from Pierrehumbert et al. 2002) includes physiological parameters defining volumes and flows in order to be able to mimic age changes. It was adapted for each chemical according to available data and toxicokinetic information.

The route of absorption by inhalation is represented in the model. In fact most of BEIs are based on a direct correlation with the TLVs which indicate the potential « inhalation » exposure of a worker. Skin exposure is not included in the models. The chemical is distributed between the central compartment and the peripheral one, or storage compartment. These compartments can be illustrated by different tissus, depending on the chemical. The

distribution can be either flow or diffusion limited and includes the permeability and affinity of the tissue for the chemical. The metabolism can give one or more metabolites, and can occur by serial or parallel metabolism. Elimination is represented by excretion in expired air, feces and urine, or by metabolism. Information on the peripheral compartment when it exits can be found in Table 2. The central compartment is then composed of the resting tissues. The model is completely described by Pierrehumbert et al. (2002). The TK model was implemented in the simulation software ITHINK ANALYST (version 7.02 for Windows, High Performance System Inc., Hanover, NH, USA).

Figure 1 Schematic diagram of the TK toxicokinetic model



Physiological and metabolic parameters

Young male physiological and metabolic parameters were taken from preceding studies and are based on reference man information (Tardif et al. 2002). These parameters, used to estimate specific data required for each chemical, were modified to take into account age based on known information about age differences. A 65-year old man has been choosen, based on the legal retirement age in Switzerland, in order to model differences due to age. Furthermore the present simulations do not take into account the apparition of various diseases with age and the increase use of medications. The main parameters required by the

model structure are body weight, cardiac output, blood flows and volumes of the relevant tissues, renal clearances and metabolic constants. The detailed list of the parameters considered is presented in Table 1.

Changes in physiological and metabolic parameters

Clewell et al. (2004) developed separate sets of growth- and age-related equations. These relations give an increase in bodyweight of 5.5 % from 25 to 65 years.

There is controversy about the effect of age on cardiac output. It has been described to decline with age (Birnbaum 1991). Clewell et al. (2004) have developed an age-related equation for cardiac output with data obtained from Åstrand (1983) and show a difference of about 10 % between 25 and 65. According to Tonner et al. (2003), cardiac output decreases in an almost linear fashion after the third decade of life, with a rate of about 1 % per year in healthy individuals without prevalent cardiac disease. This would be a difference of 30 % between 25 and 60. Fagiolino et al. (2006) indicate that cardiac output diminishes throughout life, changing 1 % every year after the age of 25. On the other hand, Rodeheffer et al. (1984) demonstrate that there is no significant decline in cardiac output at rest or during exercise in healthy adults between the ages of 25 and 79 years. However, aging does alter the mechanism by which cardiac output is maintained during exercise. Ribera-Casado (1999) insists on the fact that in healthy elderly people, cardiac output remains practically unchanged. Ferrari et al. (2003), in a review about age and cardiovascular system, show that cardiac output is preserved with age in the resting heart. Based on these last references, cardiac output was considered here to remain unchanged with age for modeling purposes.

Concerning body composition, there is a progressive reduction in total body water and lean body mass, resulting in a relative increase in body fat with age (Mangoni and Jackson 2003). According to the ICRP (1975) the body fat fraction of weight is about 11 % for a 20-year-old

man and about 30 % for a 70-year-old man. Several authors (Kyle et al. 2001, Turnheim 2003, McLean and Le Couteur 2004) mention that body fat increases by 20 to 40 % and body water decreases by 10 to 15 % in old age. For the TK models, body fat was considered to increase by 30 % with age.

Clewell et al. (2004) analyzed data on brain and liver weights as fractions of body weight for a Japanese population. A calculation shows that the variation of liver volume between 25 and 65 is about 6 %. Le Couteur and McLean (1998) show that in general the reduction of liver size with age is noted to be of the order of 25 to 35 %. After the report of the task group on reference man, liver weight decreases particularly after an age of 70 (Snyder et al., 1975). Chouker et al. (2004) have developed formulas for the estimation of liver size upon data obtained on liver weight from 728 legal autopsies analyzed with respect to gender, age, body height, body weight, body mass index, and body surface area. From these data, the decrease in liver size is about 8 % between age 25 and age 65. Finally, on base of the formulas developed by Chouker et al., a decrease of 8 % has been retained for the TK models simulations.

Several authors describe the decrease of the kidney weight with age (Löf and Johanson 1998, Mühlberg and Platt 1999, McLean and Le Couteur 2004, Geller and Zenick 2005). The aging kidney shows a decrease in weight of about 20 to 30 %, especially in the 70s and 80s (Silva 2005). Epstein (1996) mentions a decline in male kidney weight of 19%. For the TK models, a variation of 20 % has been fixed.

Renal blood flow decreases after the forth decade by about 10 % each decade (Silva 2005) and this is not related to cardiac output. A variation of 25 % was thus included in the TK models.

Age-related declines in hepatic blood flow ranging between <0.5 to 1.5 % per year have been reported (Schmucker 2001). This would produce a greater than 40 % decline between 25 and

90 years of age. According to Anantharaju et al. (2002), when comparing subjects less than 40 years to those over 65 years, there is a decrease of 35 % in hepatic blood flow. Zoli et al. (1999) found a decrease of about 20 % between subjects under 45 years and subjects between 61 and 75 years. Based on this information, a 20% reduction in hepatic blood flow was chosen for the TK models.

Clewell et al. (2004) calculate the age-specific metabolism rates by using the adult metabolism rate, the adult liver volume, the age-specific liver volume and the appropriate linearly interpolated fractional activity (gives enzyme activity as a fraction of the adult level). Different enzyme systems have been taken in account: CYP2E1, CYP1A2, CYP2C, ADH. As these fractional activities do not change between the young adult and the old adult, the changes of the metabolism rates will depend on the liver volume. For our TK models, we decided to use the same variation as for the liver volume, which is about 8 %.

The parameter which often determines renal function is the glomerular filtration rate. Clewell et al. (2004) describe the age-related differences for urinary clearance parameters by a set of equations based on data on glomerular filtration rates. The variation is about 25 % between a person of 25 years and one of 65 years. According to Epstein (1996) and Mühlberg and Platt (1999), those variations for the same ages can be about 30 %, after a widely used formula developed by Cockcroft and Gault (1976) for estimating the creatinine clearance. Berg (2006) shows similar differences by using other methods (clearances of inulin and para-aminohippurate) for evaluating the glomerular filtration rate.

The skeleton bone tissues also show some decrease with age. The loss of bone has been estimated to be about 5-10 % per decade, beginning at about 30 years of age (Snyder et al., 1975). A value has been fixed at 20 % for the simulation of a young and an old individual.

No value has been estimated for the richly perfused tissues fraction of bodyweight because of a lack of information.

Table 1 summarizes physiological and metabolic data used in the model in term of changes between 25 and 65 years. All the values of the parameters that are chemical dependent can be found in detail in Truchon et al. (2003).

Table 1 Parameters used in the TK models. Age-related changes in physiological and metabolic parameters (age 65 compared to age 25)

	Parameter	**Unit[3]**	**Value[1] Age 25 Rest 50W**	**Variation**	**Reference for the variation**
BW	Body weight	kg	70	+5.50%	Clewell et al. 2004
Qc	Cardiac output	$l/h/kg^{0.7}$	18.0 30.8	=	Ribera-Casado 1999 ; Ferrari et al. 2003
FV_{TBW}	Total body water (TBW)	l/kg	0.62	-15%	McLean and Le Couteur 2004
FV_{fat}	Fat fraction of body weight	l/kg	0.19	+30%	Kyle et al. 2001
FV_{liver}	Liver fraction of body weight	l/kg	0.026	-8%	Chouker et al. 2004
FV_{kidney}	Kidney fraction of body weight	l/kg	0.004	-20%	Epstein 1996; Silva 2005
BF_{fat}	Fat fraction of cardiac output	-	0.05 0.06	+ 30%	Clewell et al. 2004
BF_{liver}	Liver fraction of cardiac output	-	0.26 0.16	-20%	Zoli et al. 1999
BF_{kidney}	Kidney fraction of cardiac output	-	0.11 0.20	-25%	Silva 2005
k_{ur}	Urinary excretion rate	$l/h/kg^{0.82}$	1.848	-30%	Epstein 1996 ; Clewell et al. 2004
k_{cr}	Creatinine excretion rate	$\mu mol/h/kg^{0.9}$	12.06	-30%	Epstein 1996 ; Clewell et al. 2004
RC	Renal clearance	$l/h/kg^{-0.3}$	-[2]	-30%	Epstein 1996 ; Clewell et al. 2004
KM	Michaelis-Menten constant	µmol/l	-[2]	-8%	Clewell et al. 2004
VM	Michaelis-Menten maximal rate	$\mu mol/h/kg^{0.75}$	-[2]	-8%	Clewell et al. 2004

(1) Truchon et al. 2003

(2) Values are chemical dependent

(3) The reference values are adjusted to the body surface using a formula where body weight is related to the body surface when raised at a given power.

Exposure scenarios

Repeated occupational exposures were simulated at the current Threshold Limit Values (TLV) (ACGIH 2007): 8 hours per day, 5 days per week until steady-state was reached in the tissues. For the compounds having long half-lives, steady state was also reached, by adding for example an initial dose in the central compartment or by simulating the model over long exposure times. Physical workload was set at 50W during 12 hours (including the 8 hours of exposure), and at 0W the remaining of the day.

Results

Modeling age differences in biological levels

The TK model was applied to 14 chemicals, representing 21 specific biological entities. These chemicals are examples among the list of BEIs (Truchon et al. 2003). For each chemical, important physiological and metabolic parameters were identified and included in the model structure (Pierrehumbert et al. 2002). Results obtained while simulating the influence of age are summarized in Table 2 for ages 25 and 65 years.

Table 2 Tentative predictions of age-related changes in biological levels of some occupational chemicals and their metabolites (age 65 compared to age 25)

Biological indicator	**Relative change [%] in $C_{central\ comp.,blood}$ Age 65 *vs* Age 25**	**Peripheral compartment**	**Relative change [%] in $C_{peripheral\ comp.}$ Age 65 *vs* Age 25**	**Relative change [%] in $C_{metabolite\ comp.,blood}$ Age 65 *vs* Age 25**	**Relative change [%] in $C_{excretion,urine}$ Age 65 *vs* Age 25**
Arsenic	1.0				-14.5
- methylarsonic acid				11.3	-24.0
- methylarsinic acid				63.7	53.0
Cadmium	32.4	*kidneys*	17.9		10.5
Chromium (VI)	21.9	*rpt**	20.4		14.1
Cobalt	7.8	*rpt**	6.2		-11.6
2-Ethoxyethanol	6.2				
- 2-ethoxyacetic acid				38.3	34.8
Ethylbenzene	0.3	*fat*	1.8		
- mandelic acid					10.3
Fluorides	31.5	*skeleton*	35.9		22.9
Lead	25.8	*skeleton*	18.9		
Manganese	27.2	*liver*	48.2		7.0
Mercury	27.9	*kidneys*	47.5		16.7
Methyl isobutyl ketone	19.8	*fat*	17.7		44.0
Pentachlorophenol	33.5				7.3
- conjugated pentachlorophenol					10.7
Phenol	28.7				20.4
Toluene	3.2	*fat*	5.1		
- hippuric acid				29.8	13.6
- o-cresol					10.9

*rpt = richly perfused tissues

These preliminary simulations indicate in some cases considerable differences due to age. Moreover these differences appear to depend on the chemical and the biological entity considered. For some of them age does not seem to affect biological level, for others toxicokinetic calculations tend to show that age is an important determinant.

Changes are of the order of 10 to 30 % for almost half the chemicals simulated by the TK model. About a quarter of the substances shows differences less than 10 % and for the resting quarter variations are over 30 %, with two examples up to more than 50 %.

Application to biological monitoring data

Predictions presented in Table 2 can be applied to biological monitoring of exposure. Biological reference values represent biological levels that would be observed in fluids collected in healthy workers repeatedly exposed at a given reference air concentration. It can thus be expected that, due to age, different values would be observed. Table 3 presents levels that would be observed in 65 years old workers, taking young workers as reference. This information could be used to improve our interpretation of biological monitoring results, unless better information is available.

Table 3 Predicted biological reference values in 65 years old workers

Chemical/ ***biological indicator***	**Sampling time[(1)]**	**Corresponding exposure level**	**BEI[(2)]**	**VEI[(3)]**	**Simulated value Age 25**	**Simulated value Age 65**	**Expected change [%] Age 65 *vs* Age 25**
Arsenic		0.01 mg/m^3					
inorganic arsenic and its metabolites in urine	EW		35 µg As/l	14.2-66.9	63.7	84.2	32
Cadmium		0.01 mg/m^3					
cadmium in urine	NC		5 µg/g creatinine	1.6-14.9	4.7	5.6	19
cadmium in blood	NC		5 µg/l	3.8-6.5	1.2	1.7	42
Chromium (VI)		0.05 mg/m^3					
chromium in urine	ESW		25 µg/l	15.4-72.3	43.9	51.9	18
Cobalt		0.02 mg/m^3					
cobalt in urine	ESW		15 µg/l	4.7-17.9	10.4	9.8	-6
cobalt in blood	ESW		1 µg/l	0.59-1.18	0.85	0.92	8
2-Ethoxyethanol		5 ppm					
2-ethoxyacetic acid in urine	ESW		100 mg/g creatinine	41.5-244.1	68.7	94.1	37
Ethylbenzene		100 ppm					
mandelic acid in urine	ESW		0.7 g/g creatinine	0.6-3.8	1.6	1.8	13
Fluorides		2.5 mg/m^3					
fluorides in urine	PS		3 mg/g creatinine	1.2-7.9	3.4	3.5	3
fluorides in urine	ES		10 mg/g creatinine	4.5-22.4	9.4	10.6	13
Lead		0.05 mg/m^3					
lead in blood	NC		300 µg/l	161.2-561.6	188.5	217.6	15
Mercury (inorganic)		0.025 mg/m^3					
mercury in urine	PS		35 µg/g creatinine	10.6-113.6	30.8	35.1	14
mercury in blood	ESW		15 µg/l	9.2-25.1	13.6	16.7	23
Methylisobutylketone		50 ppm					
methylisobutylketone in urine	ES		2 mg/l	0.8-5	4.1	6.1	49
Pentachlorophenol		0.5 mg/m^3					
conjugated pentachlorophenol in urine	PSW		2 mg/g creatinine	0.9-4.2	1.8	2.1	17
pentachlorophenol in blood	ES		5 mg/l	2.7-10.1	3.7	4.7	27
Phenol		5 ppm					
phenol in urine	ES		250 mg/g creatinine	97.4-511.4	327.9	402.1	23
Toluene		20 ppm					
o-cresol in urine	ES		0.5 mg/l	0.16-1.53	0.98	1.09	11

(1) Time of sampling: ES = end of shift; EW = end of workweek; ESW = end of shift at end of workweek; NC = not critical; PS = prior to shift; PSW = prior to last shift of the workweek

(2) BEI = biological exposure indices (ACGIH® 2007)

(3) VEI = variability extent index with the 95% limit values (Truchon et al. 2006)

Discussion

The TK model used here is relatively simple and do not represent each chemical's specific detail. More elaborated techniques, such as PBPK models, could give a better view of the effect of age. However it would be more difficult to develop a general model for a category of chemicals describing the differences due to age.

The present results indicate that the influence of age is chemical specific. Some chemicals seem to be hardly affected while others show large differences. The same is indeed true when considering biological indicators of exposure.

For chemicals in blood, differences can be important due for example to the presence of a peripheral compartment in the toxicokinetic model. Changes due to age like the lean body fraction, the kidney fraction or the liver fraction of bodyweight could explain an increase in the blood concentration of a substance. When the peripheral compartment is represented by the volume of body fat, differences are not so important. Changes in body mass, or more specifically in total body water and body fat, will have an influence on the distribution volumes of a chemical. The consequence can be an increased concentration of hydrophilic substances and a prolonged half-life for a lipophilic substance. Products with a fast metabolism seem to have a little impact with age on the blood concentration. In the case of the concentration in the peripheral compartment, it seems to increase with age when the target organ is represented by the

liver or the kidneys. When looking at the differences due to age for the metabolic and excretion compartment, it is evident that for several chemicals, the elimination parameters as the excretion rate have a significant impact on the concentrations. In very few cases, there is a decrease in the urinary concentration of the substance when the aged person is compared to the younger one, which could be explained by the interindividual variablity. Thus it seems difficult to set up simple rules for a category of chemicals, each chemical being a specific case which has to be investigated by its own. Parameters as metabolism, storage, long half-lives, hydrophilic or lipophilic property of a chemical are often chemical-specific. It is not evident to do some classification like organic and inorganic products, or lipophilic and hydrophilic substances because in both cases important and less important differences exist.

In this context, toxicokinetic models seem to be appropriate tools. It is therefore essential to study and describe not only some specific chemicals, but also the mechanism underlying those changes in order to be able to forecast changes for new chemicals.

When compared to global biological variability expected in workers (Lin et al. 2005; Truchon et al. 2006) which is about 30 %, the tentative predictions made here indicate that age could be in some cases an important determinant of variability. The variability extent index estimated by Truchon et al. (2006) which had not taken into account variability due to age, show that in some cases, the simulated value of a 65-year old worker can be out of the estimated interval of values. It is also interesting to notice that in several cases the simulated values exceed the biological exposure indices. In very few cases, there is a decrease in the urinary concentration of the substance when the aged person is compared to the younger one. Predictions of age differences in biological indicators were made based on typical values for specific physiological and metabolic parameters. Further work should be done to improve our estimates of these parameters and in

consequence their influence. Notably uncertainty and variability in these parameters could be considered, using for example Monte-Carlo simulations. Further studies, like human volunteer exposure, need to be done to reach a better quantitative description of age differences in biological monitoring results.

Furthermore the present predictions do not take into account the apparition of various diseases with age and the increase use of medications. These aspects would probably enhance differences. The present predictions should therefore be considered as minima to be expected.

Acknowledgments We want to thank the « Agence française de sécurité sanitaire de l'environnement et du travail » in France and the « Ministère de la Culture, de l'Enseignement Supérieur et de la Recherche » as well as the « Ministère de la Santé » from Luxemburg for their financial support.

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3.2. Paper II

Sensitive headspace gas chromatography analysis of free and conjugated 1-methoxy-2-propanol in urine

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Abstract

Glycol ethers still continue to be a workplace hazard due to their important use on an industrial scale. Currently, chronic occupational exposures to low levels of xenobiotics become increasingly relevant. Thus, sensitive analytical methods for detecting biomarkers of exposure are of interest in the field of occupational exposure assessment.

1-Methoxy-2-propanol (1M2P) is one of the dominant glycol ethers and the unmetabolized urinary fraction has been identified to be a good biological indicator of exposure. An existing analytical method including a solid-phase extraction and derivatization before GC/FID analysis is available but presents some disadvantages.

We present here an alternative method for the determination of urinary 1M2P based on the headspace gas chromatography technique. We determined the 1M2P values by the direct headspace method for 47 samples that had previously been assayed by the solid-phase extraction and derivatization gas chromatography procedure. An intermethod comparison based on a Bland-Altman analysis showed that both techniques can be used interchangeably. The alternative

method showed a tenfold lower limit of detection (0.1 mg/L) as well as good accuracy and precision which were determined by several urinary 1M2P analyses carried out on a series of urine samples obtained from a human volunteer study. The within- and between-run precisions were generally about 10 %, which corresponds to the usual injection variability.

We observed that the differences between the results obtained with both methods are not clinically relevant in comparison to the current biological exposure index of urinary 1M2P. Accordingly, the headspace gas chromatography technique turned out to be a more sensitive, accurate and simple method for the determination of urinary 1M2P.

Keywords: Biological monitoring - 1-Methoxy-2-propanol - Headspace gas chromatography

1. Introduction

Since the 1970s, glycol ethers represent an interesting solvent family for industrial use due to their amphiphile behavior. They can be classified into two groups: ethylene glycol ethers (EGE) and propylene glycol ethers (PGE). Glycol ethers from the ethylenic series were used on a large scale until their reprotoxicity was discovered [1;2]. During the last twenty years, a huge work of substitution took place by proposing glycol ethers from the propylenic series. The main difference between these two groups of chemicals lies in their way of biotransformation (see [3] for a good review of the metabolism and the disposition of glycol ethers).

In humans, EGE are first transformed into 2-alkoxy acetaldehydes by alcohol deshydrogenases, and then into alkoxyacetic acids by aldehyde deshydrogenases, which are metabolites considered responsible for toxicities. PGE exist under the form of two isomers, [4] alpha-isomer and beta-isomer. Alpha-PGE are primarily metabolized through microsomal o-demethylation yielding to relatively non-toxic metabolites of propylene glycol, carbon dioxide and glucuronide as well as sulfate conjugates of the parent compound. In contrast, the beta-isomer, because it is a primary alcohol, is an excellent substrate for alcohol dehydrogenase. Metabolism of the beta-isomer is thus similar to that observed for the ethylene glycol ether series.

1-Methoxy-2-propanol (1M2P), the shorter compound from the propylenic series, is used in a wide variety of industrial and commercial products, primarily water-based paints, varnishes and inks [5-7]. Commercial grade 1M2P is a mixture of two isomers: the major form being the alpha-isomer 1M2P and the minor form being the beta-isomer 2-methoxy-1-propanol, which is considered as an impurity.

In spite of some work [8;9] showing the validity of urinary alkoxypropionic acids as biomarker of 1M2P exposure, the need to analyse unmetabolized 1M2P in urine as free or conjugated products is also becoming more interesting for the exposure assessment [10;11;12], since the conjugated 1M2P fraction appeared to be between 30% and 65% [4]. Moreover, different countries have published a biological exposure index for urinary 1M2P at the end of the work shift, fixed at 15 mg/L by the German Research Foundation [13] and 20 mg/L by the Swiss National Accident Insurance Fund [14].

In the frame of a planned study on mice exposed to 1M2P vapors (unpublished results), our former method [4] has been first modified and then replaced because of its low sensitivity due to small volumes of biological fluids. Thus, there is an obvious need to develop a sensitive, reliable and alternative method.

In this study, a GC headspace injection method is proposed to quantify free and conjugated 1M2P in urine. This analytical method has also been compared to the previous solid-phase extraction-derivatization GC procedure [4] by quantifying urinary 1M2P in about forty samples of volunteers exposed to 1M2P using both methodologies. Lastly, our analytical method was validated and applied to the analysis of urine samples collected from volunteers exposed to 1M2P.

2. Experimental

2.1 Chemicals and standards preparation

1M2P (> 98%) as analytical standard was obtained from Sigma-Aldrich (Buchs, Switzerland) and tert-butoxy-2-propanol as internal standard (IS) from Fluka (Buchs, Switzerland). All other

products, anhydrous sodium sulfate, hydrochloric acid and sodium hydroxide were obtained from Merck (Darmstadt, Germany) and were used without further purification. Water was purified through a Millipore treatment system.

The primary stock solutions were prepared by dilution of 50 µl of the commercial product in 50 mL of deionized water to obtain respective concentrations of 0.921 mg/mL for M2P and about 1 mg/mL for tert-butoxy-2-propanol used as internal standard.

Working standard of 1M2P (18.42 µg/mL) was prepared by dilution of 1.0 mL of the stock solution to 50 mL of deionized water. For the internal standard, the working solution is diluted 50 fold in deionized water. The stock solutions were kept between 5°C and 8°C and were stable for at least one month. Working solutions were freshly prepared every day.

2.2 Equipment

The apparatus used for the urine analysis was an Agilent 6890 gas chromatograph (Agilent Technologies AG, Urdorf, Switzerland) equipped with a Gerstel Multipurpose Sampler (Gerstel AG, Sursee, Switzerland) operated in headspace mode and with a 2.5 mL tight gas syringe.

The separation was performed on a CP-Sil 8 CB capillary column (95% dimethylpolysiloxane polymer, 5% phenyl groups; 60 m x 0.25 mm i.d., 0.25 □m film thickness, Varian Chrompack (Milian SA, Geneva, Switzerland)) using nitrogen as a carrier gas at a constant flow rate of 1.5 mL/min. The oven temperature was initially held at 90°C during 8 minutes, then increased to 250°C at 20°C/min and held for 2 minutes. The injector temperature was set at 250 °C and injection was performed with a split ratio of 10:1. The flame ionization detector was set at 250°C with an air flow rate of 300 mL/min, hydrogen flow rate of 30 mL/min and a nitrogen makeup flow rate of 30 mL/min.

The urine sample was kept at a temperature of 95°C during 60 minutes in the agitator of the Multipurpose Sampler. The incubation of the samples was performed automatically in the headspace mode, with a correct throughput as the agitator has 6 positions. The syringe temperature was 80°C and the injected sample volume was 1000 µl.

The retention times in these conditions were 6.81 minutes for 1M2P and 11.60 minutes for the internal standard.

2.3 Calibration and urine samples preparation

A set of seven 1M2P calibration points was prepared at the 0, 2.30¸ 4.61, 6.91, 9.21, 13.82 and 18.42 mg/L levels in water by direct addition in a 20 mL headspace flask containing 6 g of Na_2SO_4 of 0, 250, 500, 750, 1000, 1500 and 2000 µl of the 1M2P working standard solution. The final volume was then adjusted to 2 mL with deionized water. After the addition of 100 µl of the internal standard working solution, the vials were capped.

Two milliliters of human urine and 100 µl of internal standard solution were transferred to the 20 mL headspace vial, screw top, containing 6 g of Na_2SO_4. The vial was rapidly sealed with a PTFE-septum magnetic screw cap.

To determine the quantity of conjugated 1M2P, an acidic hydrolysis was realized on each urine sample. After the preparation of the calibration points and the samples by the same procedure as above, 200 µl of 10M hydrochloric acid were added to each specimen. Tubes were capped, mixed and incubated at 100°C for 16 hours. After allowing specimens to cool to room temperature, the reaction medium was neutralized by addition of 200 µl of 10M sodium hydroxide. The vials were capped and then ready for the analysis.

The difference in volume (400 µL) between the samples without hydrolysis and those with hydrolysis was insignificant in comparison to the volume of the headspace vial (20 mL). Moreover, the matrix effect was not relevant due to the addition of a large amount of salt.

2.4 Internal quality control

For assessment of assay precision, urine pools were prepared at two concentrations levels: 1.5 mg/L and 2.9 mg/L for the samples without hydrolysis process and 1.7 mg/L and 4.9 mg/L for the samples with hydrolysis process. They were aliquoted and stored at –20°C until used to assay.

3. Results

3.1 Effect of salt addition

As recommended since 1981 by Sedivec [15] and largely experimented by our team for alcohol determination in urinary samples [16], the addition of a saturating amount (6 g) of Na_2SO_4 has an important effect on the partition coefficient of an alcohol. A single experiment has been carried out, comparing a double set of five 1M2P calibration points without salt to another one with salt. In fact, as illustrated in Fig. 1, the salt addition induced a major increase of the chromatographic signal of compounds of interest with a mean improvement of 7.9±0.4 times for 1M2P and for IS. Based on these facts, analysis was performed with salt addition.

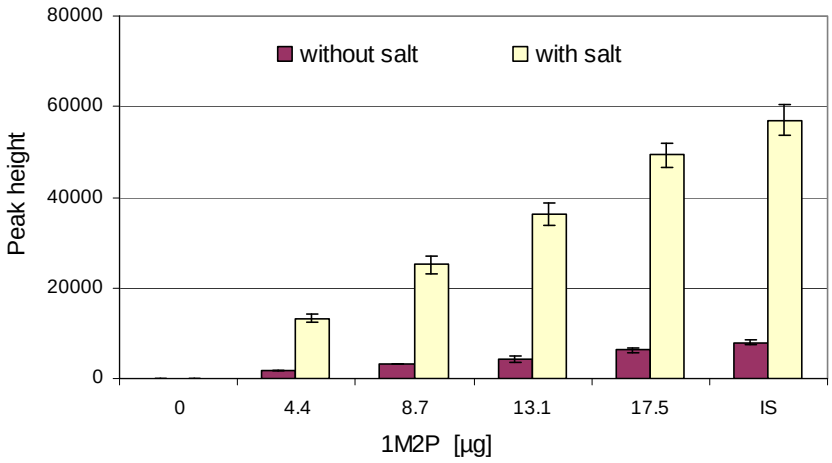

Fig. 1 Effect of addition of saturating amount (6 g) of Na_2SO_4 on spiked urine samples (calibration points) on the chromatographic response of 1M2P at different levels and on the signal of tert-butoxy-2-propanol used as internal standard (IS).

3.2 Hydrolysis conditions

While acidic hydrolysis temperature was kept as in the former method [4], the hydrolysis time has been increased. Indeed, the 1M2P peak height after 10 hours is nearly a third time higher than the one obtained after 2 hours of incubation. Samples were thus kept at 100°C overnight.

In comparison to the analyte, the internal standard has to be a non-endogenous structural analogue and preferably of low toxicity. Tert-butoxy-2-propanol has been identified as the most appropriate internal standard for the method in question. However, the tert-butyl group is easily cleaved off under strongly acidic conditions, particularly if heated, and for this reason, the IS has been added after hydrolysis.

3.3 Method-to-method comparison

For method comparison, we determined the urinary 1M2P values by the method proposed here for 47 samples that had previously been assayed by GLC method [4] and stored at –20°C in the meantime. One alternative allowing to affirm if two methods can be used interchangeably or if the new method can replace the existing one is the methodology proposed by Altman and Bland [17]. The Bland-Altman method permits in our case to determine consistency between two sets of measurements, which are here the urinary 1M2P concentrations obtained for 47 samples by two different methods [18]. The first step consisted to construct a scatter plot using the direct headspace method (GC-HS method) *vs* gas chromatography with liquid injection method after solid-phase extraction and derivatization (GLC method), with the line of perfect correlation superimposed (y=x). The results are illustrated in Fig. 2 for the urinary 1M2P concentrations determined without hydrolysis only, as the results are similar when hydrolysis takes place.

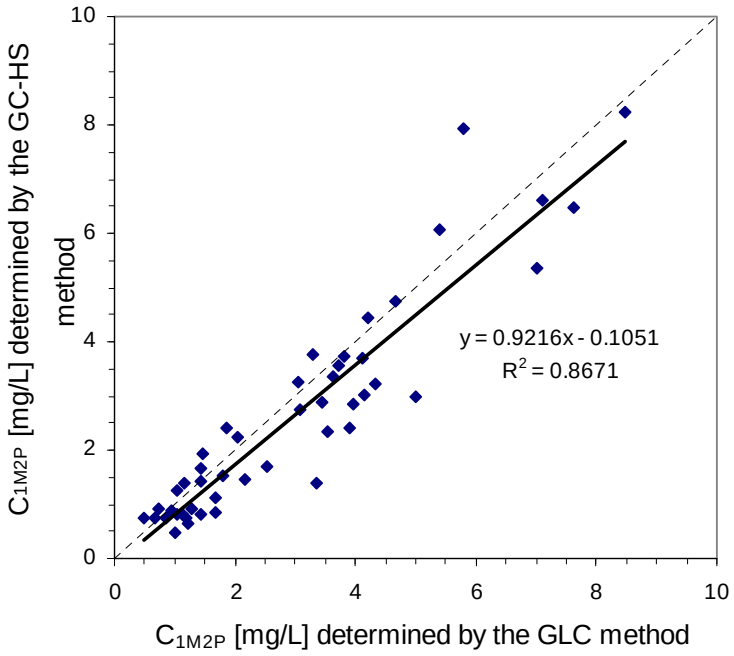

Fig. 2 Scatter plot of 1M2P concentrations (C_{1M2P}) determined without hydrolysis using the direct headspace method (GC-HS method) *vs* gas-chromatography with liquid injection method after solid-phase extraction and derivatization (GLC method [4]), with the line of perfect correlation superimposed (y=x) represented by dotted lines

A high correlation does not automatically imply that there is good agreement between the two methods. For this reason, the Altman and Bland methodology proposes to plot a graph by assigning the mean of the two measurements as the abscissa (x-axis) value, and the difference between the two values as the ordinate (y-axis) value. The Bland-Altman plots are represented on Fig. 3, with the limits of agreement represented by dotted lines (mean difference between the two methods ± 2 standard deviations of the differences). The results illustrated in Fig. 3 correspond to the urinary 1M2P concentrations determined without hydrolysis only, as the results are similar when hydrolysis takes place.

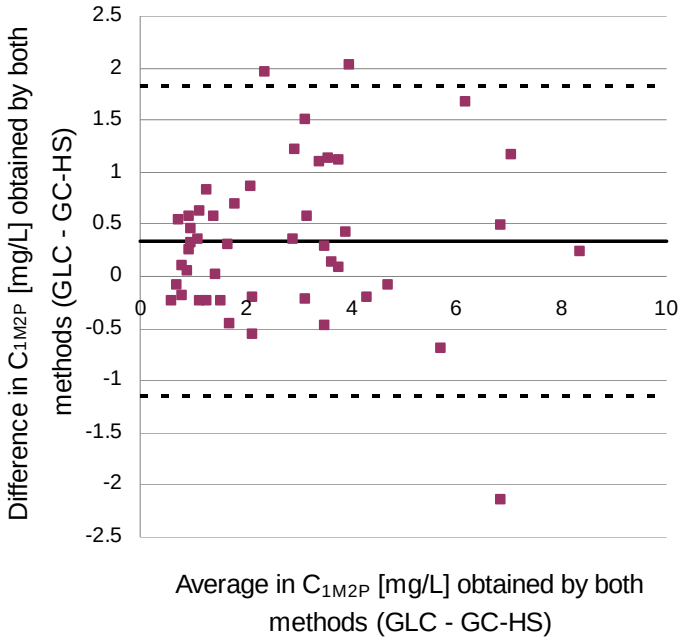

Fig. 3 Plot of difference *vs* mean (Bland-Altman plots) of 1M2P concentrations (C_{1M2P}) determined without hydrolysis using the direct headspace method (GC-HS method) and gas-chromatography with liquid injection method after solid-phase extraction and derivatization (GLC method [4]), with the limits of agreement represented by dotted lines (mean difference between the two methods ± 2 standard deviation of the differences)

3.4 Linearity, limit of detection, within- and between-run precision, accuracy

Calibration standards were prepared and analyzed in duplicate in five independent runs. The calibration curve was constructed using 1M2P/IS peak height ratios *vs* 1M2P concentration at seven levels in the range 0 to 20 µg/l. The calibration curves were linear over the entire investigated range with a correlation coefficient typically above 0.99. The analytical limit of detection (LOD) was 0.1 mg/L estimated from repeated analysis of blank specimens.

A low and a high value for the calibration on one hand and for the internal controls on the other hand have been chosen to represent within- and between-run precision, generally about 10 % which corresponds to the usual injection variability, and accuracy (99.7-106.4 %) for the determination of urinary 1M2P concentrations. The results are summarized in Tables 1 and 2, without and with hydrolysis process respectively.

Table 1 Determination of free urinary 1M2P by the headspace GC technique: accuracy, within-run and between-run precisions; they are represented by several 1M2P analyses carried out on a low and a high value of 1M2P, for the calibration on one hand and for the internal controls on the other hand.

		1M2P theoretical concentrations			
		Calibration points		Internal controls	
		2.0 [mg/L]	10.0 [mg/L]	1.5 [mg/L]	2.9 [mg/L]
Run 1	Mean *(N=4)*	2.185	10.038	1.438	2.993
	CV%*	1.8	5.0	10.5	8.1
	% Theoretical	109.3	100.4	98.5	103.2
Run 2	Mean *(N=4)*	2.342	10.067	1.435	2.935
	CV%*	6.4	2.8	9.1	2.3
	% Theoretical	117.1	100.7	98.3	101.2
Run 3	Mean *(N=4)*	2.061	10.081	1.660	2.988
	CV%*	15.6	3.4	7.4	10.3
	% Theoretical	103.1	100.8	113.7	103.0
Run 4	Mean *(N=4)*	1.920	10.100	1.288	2.693
	CV%*	8.8	14.0	8.7	14.7
	% Theoretical	96.0	101.0	88.2	92.8
Overall	Mean *(N=16)*	2.127	10.0.71	1.455	2.902
	CV%**	11.2	7.0	12.4	9.7
	% Theoretical***	106.4	100.7	99.7	100.1

with N = number of samples
CV = coefficient of variation

* Within-run precision
** Between-run precision
*** Accuracy

Table 2 Determination of total urinary 1M2P by the headspace GC technique: accuracy, within-run and between-run precisions; they are represented by several 1M2P analyses carried out on a low and a high value of 1M2P, for the calibration on one hand and for the internal controls on the other hand.

		1M2P theoretical concentration			
		Calibration points		Internal controls	
		2.0 [mg/L]	10.0 [mg/L]	1.7 [mg/L]	4.9 [mg/L]
Run 1	Mean *(N=4)*	1.790	10.186	1.533	4.613
	CV%*	5.1	4.9	6.3	1.5
	% Theoretical	89.5	101.9	90.1	94.9
Run 2	Mean *(N=4)*	2.066	10.544	1.675	5.063
	CV%*	12.7	7.8	7.5	5.2
	% Theoretical	103.3	105.4	98.5	104.2
Run 3	Mean *(N=4)*	1.955	10.192	1.728	4.873
	CV%*	10.4	7.4	10.8	10.1
	% Theoretical	97.7	101.9	101.6	100.3
Run 4	Mean *(N=4)*	2.209	10.712	1.845	4.873
	CV%*	8.2	11.1	6.6	12.3
	% Theoretical	110.5	107.1	108.5	100.3
Overall	Mean *(N=16)*	2.005	10.409	1.695	4.855
	CV%**	11.7	7.7	10.0	8.3
	% Theoretical***	100.2	104.1	99.7	99.9

with N = number of samples
CV = coefficient of variation

* Within-run precision
** Between-run precision
*** Accuracy

4. Discussion

The results of our study suggest that urinary 1M2P can be quantified by a GC headspace injection method, which is especially useful for the assessment of low occupational exposure levels.

For 1M2P, with a vapor pressure of 12 mmHg (1.60kPa), the headspace technique, as applied to different organic volatiles [15], should be suitable to extract this methoxy alcohol derivative from biological fluid..

In comparison to the existing analytical method [4], several aspects have been improved. The influence of the salt addition on the partition coefficients in gas-water systems with the enrichment of compounds of interest in the “head space” phase is a crucial factor in improving the sensitivity of analysis. The LOD obtained in the described conditions were much lower (10 fold better) than those obtained with the existing method for the determination of urinary 1M2P including a solid-phase extraction and derivatization before GC/FID analysis. With a LOD of 0.1 mg/L, the GC headspace technique allows to determine the target substance in small volumes of biological fluids and/or in low occupational exposures situations.

The method-to-method comparison shows on one hand that the correlation between the measured 1M2P values by both methods seems to be better for the low values as for the higher ones. The fact that there are more samples for the lower values can partly explain this observation. On the other hand, the difference against mean diagram shows that generally, the values are slightly higher for the liquid injection method as for the GC headspace technique. As a good stability of the analyte has been demonstrated by [11] and as the sample preparation is a simple procedure, the important difference in sensitivity of both methods could explain the observed variability in the measurement.

The Bland-Altman plots show that 95% of the differences obtained between both analytical methods fall within the limits of agreement. Furthermore, we can assume that these differences are not clinically relevant in comparison to the usual biological exposure indices of urinary 1M2P. Thus both methods can be used interchangeably.

The precision of the method, calculated as intra- and inter-day reproducibility, was generally about 10 %, which corresponds to the usual injection variability. The difference between theoretical concentration values and the measured one shows a high accuracy. Consequently, the important criteria for the validation of an analytical method are fulfilled.

Another advantage concerns the sample preparation, an uncomplex and short procedure thus limiting possible sources of errors due to manipulation.

Some limitations of the method exist: it is necessary to avoid the contamination of the urine samples by surrounding solvents or the loss of the analyte during manipulation and analysis.

In summary, we conclude that the determination of urinary 1M2P by the headspace technique is a high sensitive and a particularly simple analytical method, useful in the assessment of low occupational exposure levels to 1M2P. The analytical procedure could be adapted for the determination of other substances of the glycol ethers family as long as physico-chemical properties allow it.

Acknowledgments

The present work was supported by grants from the French Agency of Environmental and Occupational Health Safety (Afsset), the Swiss Federal Office of Public Health (FOPH) and the National Research Fund (FNR) in Luxembourg. We also want to express our thanks to Ms C. Arnoux for her skillful technical assistance.

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3.3. Paper III

Sex differences in urinary levels of several biological indicators of exposure: a human volunteer study.

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Abstract

The aim of the study was to quantify the variability on biological indicators of exposure between men and women for three well known solvents: methyl ethyl ketone, 1-methoxy-2-propanol and 1,1,1-trichloroethane. Another purpose was to explore the effect of selected CYP2E1 polymorphisms on the toxicokinetic profile.

Controlled human exposures were carried out in a 12 m^3 exposure chamber for each solvent separately, during 6 hours and at half of the threshold limit value. The human volunteers groups were composed of ten young men and fifteen young women, including ten women using hormonal contraceptive.

An analysis of variance mainly showed an effect on the urinary levels of several biomarkers of exposure among women due to the use of hormonal contraceptive, with an increase of more than

50 % in metabolites concentrations and a decrease of up to 50 % in unchanged substances concentrations, suggesting an increase in their metabolism rate. The results also showed a difference due to the genotype CYP2E1*6, when exposed to methyl ethyl ketone, with a tendency to increase CYP2E1 activity when volunteers were carriers of the mutant allele.

Our study suggests that not only physiological differences between men and women but also differences due to sex hormones levels can have an impact on urinary concentrations of several biomarkers of exposure. The observed variability due to sex among biological exposure indices can lead to misinterpretation of biomonitoring results. This aspect should have its place in the approaches for setting limits of occupational exposure.

Keywords: Human biomonitoring – Biological indicators of exposure – Sex differences – Toxicokinetics – Organic solvents

1. Introduction

Due to their chemical properties, organic solvents are commonly used worldwide in different industrial sectors as degreasing and diluting agents. They can be mainly classified as aliphatic hydrocarbons, cyclic hydrocarbons, aromatic hydrocarbons, halogenated hydrocarbons, ketones, amines, esters, alcohols, aldehydes, ethers and glycol ethers. Their production and use increased mainly in the latter half of the 19th century due to the coal-tar industry.

During the 1980's, a major concern for occupational hygienists and physicians was the risk assessment of occupational exposure to volatile organic compounds (Droz et al., 1983) as their neurotoxicity represented one of the most important emerging issues in the field of occupational health (Baker 1988).

Biological monitoring is a widely used method in the assessment of occupational exposure. It often relies on measurements of the chemical or its metabolites in biological fluids, identified as biomarkers of exposure. Recommended biological limits for chemical substances, most based on a direct correlation with the corresponding threshold limit values, are published periodically in Switzerland and in other countries for interpretation and decision-making.

Recently, much more attention has been devoted to variability associated with biological monitoring results (Tardif et al., 2002; Pierrehumbert et al., 2002; Truchon et al., 2006; Thomson et al., 2010). Several studies in volunteers and with toxicokinetic models have now shown that biological results follow approximatively log-normal distributions, characterized by geometric standard deviations ranging from about 1.3 to 2.0 (Droz, 1992), equivalent to a coefficient of variation between 30 % and 100 %. This large biological variability was already obvious to drug therapists (Meibohm et al., 2002; Schwartz, 2003), and adaptation of dosage is normal current practice, at least for drugs with narrow therapeutic ranges.

Among others, sex is an easily identifiable determinant which could provide an important contribution to biological variability. The influence of sex on toxicokinetics may involve female-male differences in physical constitution (body water space, muscle mass, body fat, and blood flow), physiology (menopause and menstruation cycle), hormones (contraceptive pill) and metabolising enzymes (Löf et al., 1998). Lipophilic substances will tend to have a greater distribution volume in women, hydrophilic substances will show the opposite (Schwartz, 2003). The binding in plasma to proteins is also influenced by sex hormones, which can lead to toxicokinetic differences (Gandhi et al., 2004). Cytochrome P450 dependent mono-oxygenases are the most prominent enzymes involved in solvent metabolism. Isoenzymes belonging to the first four gene families (CYP 1-4) are involved in the metabolism of xenobiotics (Löf et al., 1998). The metabolism of many solvents includes oxidation by the CYP2E1 (Nakajima et al., 1997). This specific isoform, probably among others, is known to exhibit sex differences and to show a polymorphism (Bebia et al, 2004).

There are only a few studies evaluating possible sex differences in the toxicokinetics of occupational chemicals in humans. Swedish researchers exposed male and female volunteers to m-xylene and 2-propanol under controlled conditions (Ernstgård et al., 2003a,b) and found toxicokinetic modifications for both chemicals, which they attributed mainly to anatomical differences between sexes. Small changes in acute health effects were also identified in these experiments (Ernstgård et al, 2002).

Although one can establish a list of contributing factors (Gochfeld, 2007), their relative importance for different chemicals of occupational interest is unknown. Several authors recently recommended that more toxicokinetic studies be undertaken to better characterize the influence of sex (Arbuckle, 2006; Messing, 2006).

The main objective of this study was therefore to quantify the variability on biological indicators of exposure between men and women for three well known solvents, which are methyl ethyl ketone, 1-methoxy-2-propanol and 1,1,1-trichloroethane. Another purpose was to explore the effect of selected CYP2E1 polymorphisms on the toxicokinetic profile. Some of the results of this study have been previously reported in the form of an abstract (Tomicic et al., 2010).

2. Material and methods

2.1. Human volunteers

The experimental protocol was approved by the Clinical Research Ethics Committee of the Faculty of Biology and Medicine at the University of Lausanne, according to the Declaration of Helsinki.

Ten men and fifteen women participated in the study. Each volunteer had to undergo a medical examination including a health questionnaire, a general physical examination, standard clinical blood and urine analyses, an electrocardiogram and a spirometry. Body fat was measured the day of exposure with a body fat monitor using bioelectric impedance (Omron BF 300, Omron Healthcare Europe).

The following inclusion criteria were used to select the participants: caucasian, age between 20 and 25 years, body mass index between 18 and 28, non-smoker, no history of chronic or allergy disease, no occupational exposure to organic solvents, no chronic use of drugs and no pregnancy.

2.2. Chemicals

Three chemicals have been selected for their relatively low toxicity as well as their metabolic and physiological properties: methyl ethyl ketone (MEK), watersoluble and highly metabolized, 1-methoxy-2-propanol (1M2P), amphiphile and highly metabolized, and 1,1,1-trichloroethane, liposoluble and poorly metabolized.

MEK (> 99.5 %) and 1M2P (> 98 %) were obtained from Sigma-Aldrich (Buchs, Switzerland) and 111TCE (> 99.5 %) from Merck (VWR International AG, Schlieren, Switzerland).

The three solvents are mainly absorbed by inhalation and metabolized via the cytochrome P450 mixed-function oxidase system.

MEK is metabolized to 2,3-butanediol, 2-butanol and 3-hydroxy-2-butanone (major metabolite), all excreted in urine. A low fraction of unchanged solvent is excreted in the exhaled air and in urine. The urinary concentration of MEK at the end of the shift has been recommended as the most appropriate biological exposure indicator.

1M2P belongs to the family of the propylene glycol ethers (PGE) which exists under the form of two isomers, alpha-isomer and beta-isomer. The latter one, considered as an impurity in commercial use, is first transformed as all primary alcohols into 2-alkoxy acetaldehydes by alcohol deshydrogenases, and then into alkoxyacetic acids by aldehyde deshydrogenases, a toxic metabolite. Alpha-PGE are primarily metabolized to propylene glycol, carbon dioxide and glucuronide as well as sulfate conjugates of the parent compound. The urinary 1M2P concentration at the end of the shift corresponds as the appropriate biomarker.

1,1,1-trichloroethane is metabolised to trichloroethanol (TCE) and trichloroacetic acid (TCA), both excreted in urine. Other minor metabolites (carbon dioxide, acetylene) are excreted in the

exhaled air. The corresponding biological exposure indices are the urinary concentration of both metabolites and the blood concentration of the parent compound. The 111TCE concentration in the exhaled air can also be considered as a biomarker of exposure.

Table 1 summarizes useful information about existing threshold limit values and biological exposure indices regarding the biological determinants corresponding to the three studied organic solvents.

Table 1. Summary of the selected organic solvents with occupational exposure limits (OELs in ambient air) and corresponding biomarkers of exposure,for different countries (USA, Germany, Switzerland)

		BEI*/BAT/VBT***		
Biological determinant	**TLV*/MAK**/VME*** (ppm)**	***urine* (mg/l)**	***blood* (µg/l)**	***expired air* (ppm)**
Methyl ethyl ketone (MEK)	200/200/200	2/5/5 (ES[a])	-	-
1-Methoxy-2-propanol (1M2P)	100/100/100	-/15/20 (ES)	-	-
1,1,1-Trichloroethane (111TCE)	350/200/200	-	-/550/550 (PS[c], EW)	40/-/- (PS, EW)
Trichloroethanol (TCE)		30/-/- (ES, EW[b])	-	-
Trichloroacetic acid (TCA)		10/-/- (EW)	-	-

* The American Conference of Industrial Hygienists (ACGIH) sets threshold limit values (TLV) and biological exposure indices (BEI).

** The German Research Foundation (Deutsche Forschungsgemeinschaft - DFG) sets "Maximale Arbeitsplatz-Konzentrationen" (MAK) and "Biologische Arbeitsstoff-Toleranzen" (BAT).

*** The Swiss National Accident Insurance Fund (Schweizerische Unfallversicherungsanstalt - Caisse nationale suisse d'assurance en cas d'accidents - Istituto nazionale svizzero di assicurazione contro gli infortuni - SUVA) sets "valeurs (limites) moyennes d'exposition" (VME) and "valeurs biologiques tolérables" (VBT).

[a] ES: end of shift [b] EW: end of week [c] PS: prior to shift

2.3. Exposure conditions and experimental design

The experiments were carried out in a 12 m^3 air-conditioned exposure chamber and were conducted over 37 separate sessions. The chamber allows the simultaneous exposure of three volunteers to vapors. The responsible physician's main duties were to check the participant before exposure, to make sure that inclusion criteria were still respected, and to see the volunteer after and the day following exposure to assess any health outcome due to the exposure session. Exposures lasted for 6 hours at rest, at half of the threshold limit value of the used solvent. The intended exposure levels were based on the Swiss OELs and corresponded to 100 ppm for MEK and 111TCE, and to 50 ppm for 1M2P. Each volunteer was separately exposed to the three solvents, with a minimum of two weeks (based on a 100 hr half-time) between 2 exposure scenarios to avoid cross-cumulation. The temperature in the chamber was maintained at about 24 °C and the air renewal was between 12 and 15 per hour. A peristaltic pump pushed the solvent to a heated aluminium plate (kept at a temperature about 10 °C above the boiling point of the considered solvent) where it evaporated to be immediately injected into the ventilation of the chamber. The solvent generation was controlled by the LabVIEW software (National Instruments Corporation, Texas, USA).

2.4. Air monitoring

The solvent concentration in the chamber was continuously monitored by two independent methods: a portable FTIR (Fourier Transform Infrared Spectroscopy) gas analyzer for ambient air analysis (Gasmet™ DX4015, Gasmet Technologies Oy, Helsinki, Finland) and a Perkin Elmer gas chromatograph (GC), with a flame ionization detector (FID), connected to a Hewlett Packard

3395 integrator. With the latter one, a measurement was taken every half an hour. Solvent concentration in the exposure chamber was additionally assessed by active personal air sampling. Solvents were collected on tubes containing activated charcoal as adsorbent, with air samplings rates of 40 ml/min. After desorption with carbon disulfide (in the case of MEK and 111TCE) or dichloromethane (in the case of 1M2P), analysis were done by GC using a flame ionization detector.

2.5. Biological monitoring

Sampling: Urine samples were collected before, during (with a predefined timing and in a solvent-free area) and after (collected *ad lib*) exposure. The samples were kept in polyethylene bottles or in brown borosilicate flasks directly after reception and stored refrigerated at 4 °C. When kept in polyethylene bottles, samples were aliquoted and stored frozen (-20 °C) at the end of the exposure session. Blood samples were taken before and after the exposure, as well as during the following day when the volunteers came back to bring the urine samples. Venous blood was collected in 9-ml vacuum tubes with an anticoagulant comprising ethylenediaminetetraacetic acid (EDTA) and stored in a refrigerator at 4 °C until analysis. Expired air was sampled immediately after the exposure by taking at least 3 points during the hour following the exposure.

Analytical methods: Analytical methods for exposure measurements and biological determinations in urine, blood and expired air are mostly under control at the Institute for Work and Health (IST) in Lausanne, Switzerland, in an ISO 17025 certification scheme. Parent compounds and metabolites have been analysed by gas chromatography according to published schemes. The determination of all the defined biomarkers of exposure was based on the

headspace gas chromatography technique. As recommended since 1981 by Sedivec et al. (1981) and largely experimented by our team for alcohol determination in urinary samples (Sethre et al., 2000), the addition of a saturating amount of Na_2SO_4 has an important effect on the partition coefficient of solvents, in our case of MEK and 1M2P. An existing analytical method for the determination of urinary 1M2P including a solid-phase extraction and derivatization before analysis is available (Devanthéry et al., 2000) but presents some disadvantages. In the frame of this study, an alternative method based on the headspace gas chromatography technique has been developed (Tomicic et al., 2010). The quantity of conjugated 1M2P has been determined after an acidic hydrolysis, realized by adding hydrochloric acid to the samples and by keeping them at 100°C overnight.

Regarding the 111TCE metabolites, urinary TCE and urinary TCA have been analysed after methylation, transforming TCA to its corresponding and more volatile ester, and after hydrolysis, in order to assess total TCE as it can be present under its conjugated form (Breimer et al., 1974).

As recommended for spot urine specimen, creatinine adjustment was performed for each sample to correct for urine dilution. Creatinine concentration was determined by a modified Jaffe reaction using a deproteinisation before the addition of picric acid. The colorimetric intensity of the creatinine-picrate complex was measured at 520 nm. Normal creatinine values are between 0.3 and 3.0 g/l.

The apparatus used for the urine analysis was an Agilent 6890 gas chromatograph (Agilent Technologies AG, Urdorf, Switzerland) equipped with a Gerstel Multipurpose Sampler (Gerstel AG, Sursee, Switzerland) operated in headspace mode and with a 2.5 mL tight gas syringe. The flame ionization detector (FID) has been used for the determination of the alcohols and the micro electron capture detector (μ-ECD) in the case of the chlorinated compounds.

The analyses of the solvents in the end-expired air were performed by using a specially designed sampling valve (Droz et al., 1986; Devanthéry et al., 2002), linked to a carbon dioxide detector and using a Perkin Elmer GC connected to a Hewlett Packard 3395 integrator.

2.6. Identification of genotypes

The most studied genetic variants CYP2E1*5B and CYP2E1*6 (Prieto-Castelló et al., 2010) were explored. Genotypes of the 25 participants have been identified by using PCR-based RFLP methods, as described by Prieto-Castelló et al. (2010). DNA extracted from blood was amplified with specific primers (QIAamp DNA Kit, Qiagen).

The primers used for CYP2E1*5B are described by Cai et al. (2005) and digestion occured during 3 hours at 37 °C with Pst I. Non-digested 553 bp fragments represented c1/c1 wild-type allele, fragments of 435 + 118 bp represented the c2/c2 genotype and fragments of 553 + 435 + 118 bp represented the c1/c2 genotype.

The primers used for CYP2E1*6 are described by Prieto-Castelló et al. (2010) and digestion was carried out during 3 hours at 37 °C with Dra I. Non-digested 373 bp fragments represented homozygous AA, fragments of 249 + 124 bp represented homozygous wild-type TT and fragments of 373 + 249 + 123 bp represented heterozygous TA.

2.7. Symptom questionnaire

No side effects are expected under these exposure conditions. However, irritation and central nervous system effects typical of some solvent exposures cannot be excluded. These possible symptoms were self-reported by the volunteers by filling a standard symptom questionnaire at

different exposure times. The questionnaire has been taken from a Swedish study (Ernstgård et al., 2002), where the ratings were performed using a 0-100 mm visual analogue scale, graded from “not at all” (corresponding to 0 mm) through “hardly at all” (6 mm), “somewhat” (26 mm), “rather” (48 mm), “quite” (71 mm), “very” (90 mm) to “almost unbearable” (100 mm). The symptoms asked for were: discomfort in the eyes, in the nose or in the throat or airways, breathing difficulty, solvent smell, headache, fatigue, nausea, dizziness and feeling of intoxication.

2.8. Statistical analysis

The data were first analysed for normality using the Shapiro-Wilk test. Most observations were log normally distributed. Besides several studies in volunteers and with toxicokinetic models showed that biological results follow approximate lognormal distributions (Droz, 1992). Statistical calculations were therefore performed after logarithmic transformation of the dependent variables.

Statistical comparisons between mean values obtained at the end of exposure were made by using the Student’s two-sided t-test. The F-test was used to compare variances between groups.

Univariate analysis of covariance (ANCOVA) was also performed in order to identify potential confounders (body mass index, body fat, genotype). Correlations between mean urinary concentrations and potential confounders were evaluated by using the Pearson correlation test. The significance level was set at $\alpha=0.05$ and statistical analyses were performed using the software package STATA Version 10.

Ratings obtained from the symptom questionnaires were not normally distributed. Therefore, the Mann-Whitney U test was used to test for differences in symptom ratings between men and women.

3. Results

3.1. Human volunteers

Among all volunteers, only one woman with hormonal contraceptive declined to participate to her last exposure session, which concerned 111TCE. The characteristics of each group of human volunteers are shown in Table 2. There were no statistically significant differences in age and body mass index between the different groups. Body weight and body height were higher for men while body fat was higher in women (men *vs* women with hormonal contraceptive: $p = 0.02$, $p < 0.001$ and $p = 0.001$, respectively ; men *vs* women without hormonal contraceptive: $p = 0.01$, $p < 0.001$ and $p < 0.001$, respectively). When comparing women with hormonal contraceptive to those without, the only statistically significant difference was for body fat ($p = 0.008$).

Table 2. Characteristics of each volunteer group. Arithmetic mean values (± SD: standard deviation) are indicated; n = number of volunteers.

	Men (n=10)	**Women (n=15)**	***Women with hormonal contraceptive (n=10)***	***Women without hormonal contraceptive (n=5)***
Age (years)	22.4 ± 2.2	23.2 ± 2.9	22.6 ± 2.1	24.4 ± 4.2
Body weight (kg)	70.7 ± 10.3	61.9 ± 8.7	60.1 ± 7.2	65.6 ± 11.2
Body height (cm)	177.5 ± 3.2	165.8 ± 3.7	167 ± 3.8	163.4 ± 2.3
Body Mass Index (-)	22.3 ± 2.6	22.5 ± 3.2	21.6 ± 2.6	24.5 ± 3.7
Body fat (% of body weight)	11.3 ± 5.2	22.1 ± 6.9	19.8 ± 4.6	26.6 ± 8.9

3.2. Air monitoring

The air concentrations of the selected solvents in the exposure chamber remained very stable over time. Slight variations in solvent air concentrations were possible when volunteers went out of the chamber for urine sampling. But these variations had little impact on the mean air concentrations as the chamber door stayed open for very short moments. All methods of air monitoring agreed within 5 %. Air sampling using the tubes containing activated charcoal as adsorbent gave following solvents level: 99.15 (± 5.29) ppm for MEK, 53.22 (± 3.04) ppm for 1M2P and 102.55 (± 3.19) ppm for 111TCE, which corresponded to the intended ones.

3.3. Biological monitoring

Figures 1-4 show the mean urinary concentrations of the different biomarkers of exposure over time for the three groups of human volunteers. Toxicokinetic profiles were similar among all groups of volunteers, with differences in the maximal concentration reached. Urinary levels of biomarkers of exposure measured among women with hormonal contraceptive were in general close to the ones measured among men. Moreover urinary levels were higher in women without hormonal contraceptive when biomarkers of exposure corresponded to the unchanged substances and the opposite was observed when biological indicators of exposure corresponded to the metabolites.

During exposure, urine was collected every 2 hours. In general, mean values were calculated from concentrations obtained for each human volunteer but as urine samples have been collected *ad lib* after exposure, a reduced number of volunteers contributed to the mean urinary levels at 8, 10, 12 and 14 hours from the beginning of the exposure (for details see captions of Figures 1-4).

For urinary MEK and urinary 1M2P, adjustment to one g of creatinine was not necessary due to

their passive process of elimination in the kidney. In this case compounds secretions vary with urine flow rate and creatinine adjustment would not correct for urine dilution. Rather, in the case of urinary TCE and urinary TCA, corrections by creatinine gave less variability in the toxicokinetic profiles, showing that compensation to urine dilution was necessary.

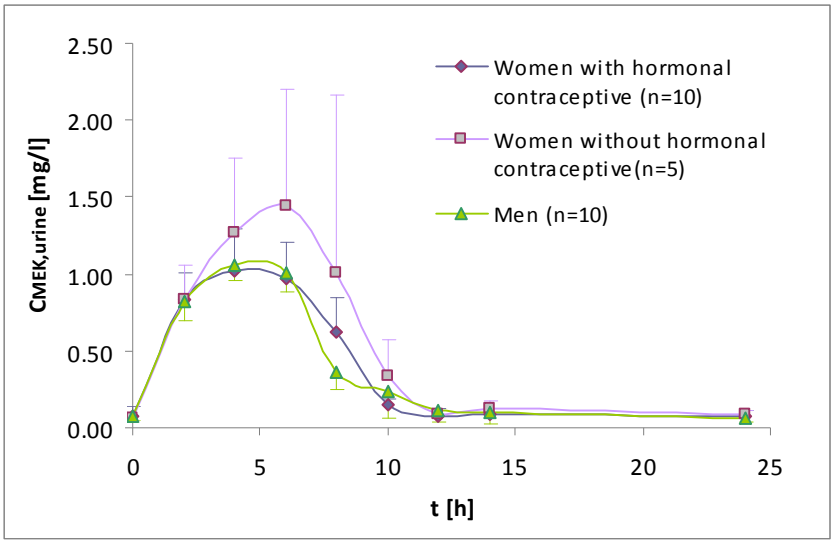


Figure 1 : Urinary MEK concentration as function of time after 6 hours of exposure to 99.15 (± 5.29) ppm MEK. Arithmetic mean values (± SD: standard deviation) are indicated; n = number of volunteers.

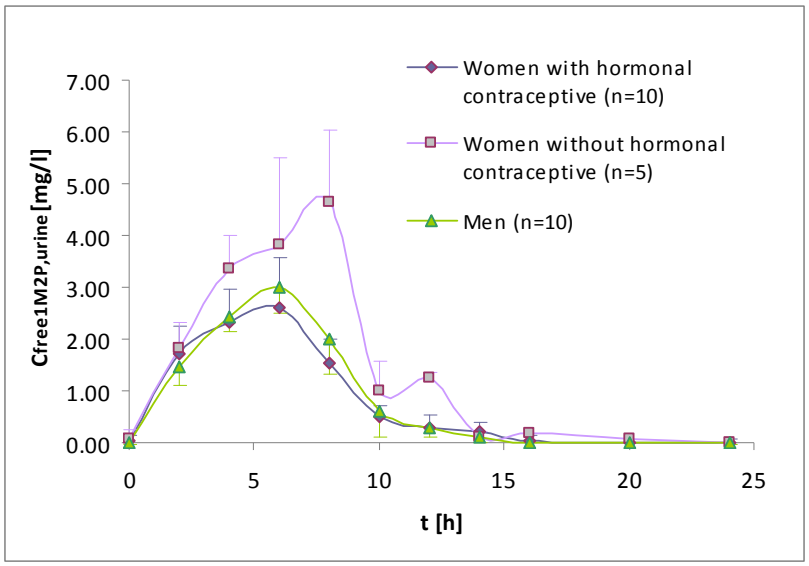
Mean urinary levels at 8, 10, 12 and 14 hours from the beginning of the exposure with:

n = 4, 6, 3 and 6 for women with hormonal contraceptive,

n = 4, 2, 1 and 4 for women without hormonal contraceptive,

n = 5, 6, 5 and 6 for men.

(a)



(b)

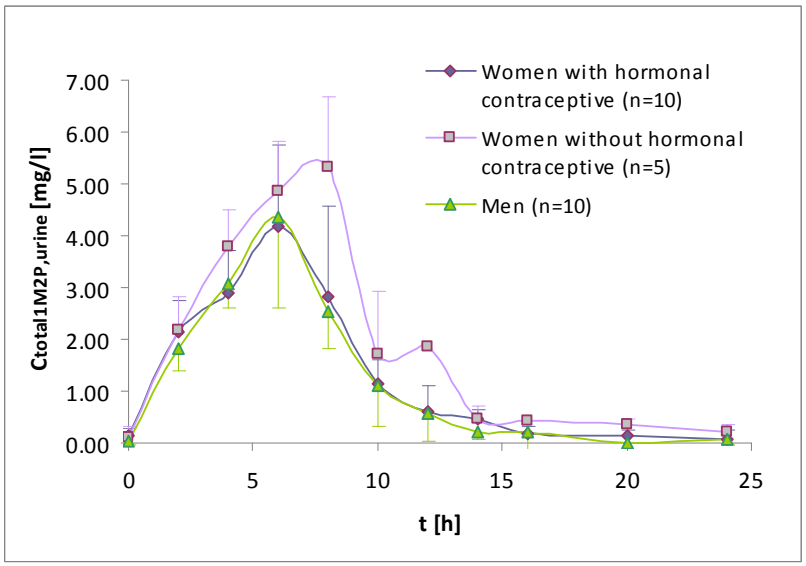


Figure 2 : Urinary free 1M2P (a) and urinary total 1M2P (b) concentrations as function of time after 6 hours of exposure to 53.22 (± 3.04) ppm of 1M2P. Arithmetic mean values (± SD: standard deviation) are indicated; n = number of volunteers.

Mean urinary levels at 8, 10, 12 and 14 hours from the beginning of the exposure with:

n = 4, 8, 5 and 3 for women with hormonal contraceptive,

n = 3, 4, 1 and 2 for women without hormonal contraceptive,

n = 6, 4, 7 and 5 for men.

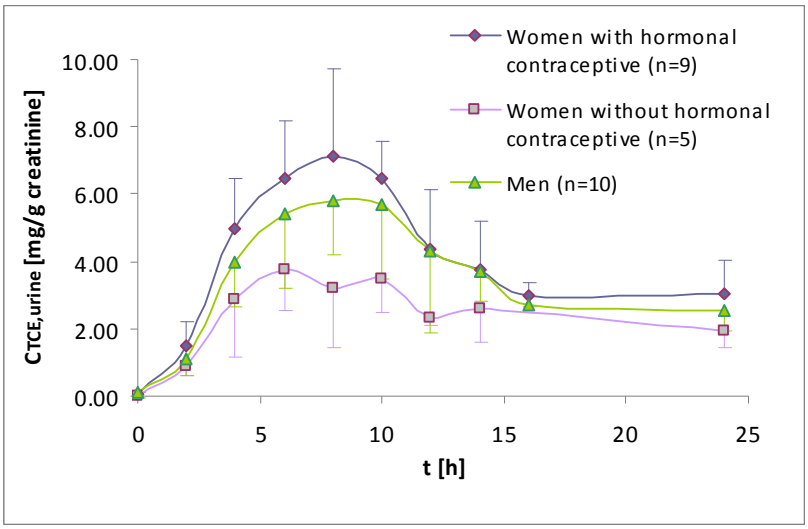


Figure 3 : Urinary TCE concentration as function of time after 6 hours of exposure to 102.55 (± 3.19) ppm of 111TCE. Arithmetic mean values (± SD: standard deviation) are indicated; n = number of volunteers.

Mean urinary levels at 8, 10, 12 and 14 hours from the beginning of the exposure with:

n = 5, 3, 5 and 6 for women with hormonal contraceptive,

n = 2, 4, 2 and 4 for women without hormonal contraceptive,

n = 4, 5, 7 and 3 for men.

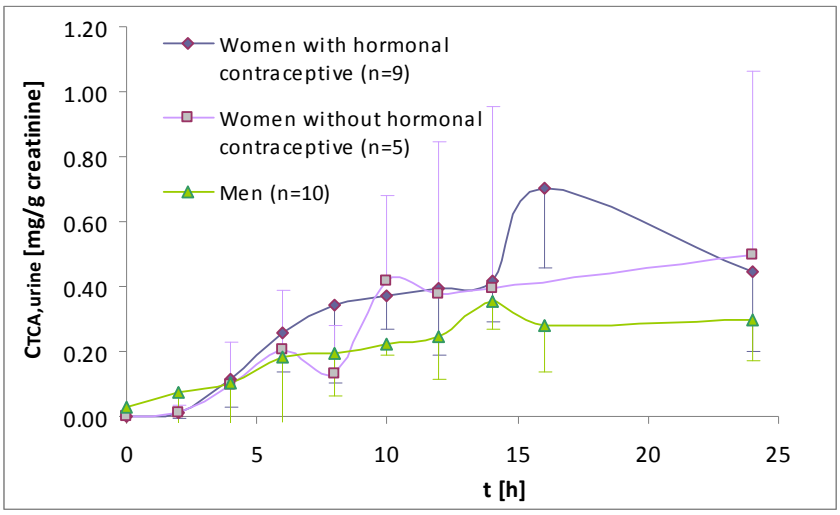


Figure 4 : Urinary TCA concentration as function of time after 6 hours of exposure to 102.55 (± 3.19) ppm of 111TCE. Arithmetic mean values (± SD: standard deviation) are indicated; n = number of volunteers.

Mean urinary levels at 8, 10, 12 and 14 hours from the beginning of the exposure with:

n = 5, 3, 5 and 6 for women with hormonal contraceptive,

n = 2, 4, 2 and 4 for women without hormonal contraceptive,

n = 4, 5, 7 and 3 for men.

End-exposure levels for the different urinary biomarkers of exposure, expressed as mean values (± SD), are summarized in Table 3. Student's tow-tailed t-tests showed statistically significant differences between women with and without hormonal contraceptive for urinary MEK, urinary free 1M2P and urinary TCE. For the latter one, statistically significant differences were also observed between men and women without hormonal contraceptive. Differences in urinary TCA levels were statistically significant between men and women with hormonal contraceptive. From the levels of free (aglycone) and total urinary 1M2P, we can deduce that urinary 1M2P is present up to 60 % in the conjugated form.

111TCE blood concentrations were determined and showed high interindividual variability for all the groups. Before exposure, values obtained for men, women with and without hormonal contraceptive, respectively were 0.60 (± 0.44), 1.00 (± 0.75) and 0.58 (± 0.33) μg/l, end-exposure levels were 362.97 (± 91.05), 306.53 (± 152.27) and 371.13 (± 240.64) μg/l and measured values at the following morning were 16.03 (± 7.71), 10.77 (± 4.46) and 17.86 (± 10.19) μg/l.

Measurements in exhaled air showed that for MEK and 1M2P, mean end-exposure levels were very low (about 2 to 3 % of the exposure concentration) with no statistically significant differences between the different groups.

In the case of exhaled 111TCE, mean end-exposure levels corresponded to 27.31 (± 11.09), 28.28 (± 10.36) and 27.47 (± 6.93) ppm for men, women with and without hormonal contraceptive, respectively. The following day, values dropped to 1.98 (± 3.11), 0.69 (± 0.27) and 0.88 (± 0.25) ppm.

Table 3. Summary of the different biomarkers of exposure values obtained at the end of exposure. Arithmetic mean values (± SD: standard deviation) are indicated; n = number of volunteers.

Biological indicator	**Men (n=10)**	**Women with hormonal contraceptive (n=10)**	**Women without hormonal contraceptive (n=5)**	**p-value**
Methyl ethyl ketone				
Urinary MEK at the end of exposure (mg/l)	1.00 ± 0.13	0.97 ± 0.23	1.44 ± 0.76	ns[*]/ns[**]/0.09[***]
1-Methoxy-2-propanol				
Free urinary 1M2P at the end of exposure (mg/l)	2.99 ± 0.49	2.61 ± 0.96	3.81 ± 1.68	ns/ns/0.1
Total urinary 1M2P at the end of exposure (mg/l)	4.36 ± 1.76	4.17 ± 1.58	4.85 ± 0.97	ns/ns/ns
Mean percentages of conjugated 1M2P (%)	26.62 ± 16.77	31.18 ± 34.16	22.70 ± 22.75	ns/ns/ns
1,1,1-Trichloroethane				
Urinary TCE at the end of exposure (mg/l)	5.66 ± 2.20	3.83 ± 1.26	2.09 ± 1.24	ns/0.01/0.03
Urinary TCE at the end of exposure (mg/g creatinine)	5.42 ± 2.19	6.46 ± 1.73	3.77 ± 1.24	ns/0.08/0.01
Urinary TCA at the end of exposure (mg/l)	0.20 ± 0.23	0.16 ± 0.08	0.11 ± 0.09	ns/ns/ns
Urinary TCA at the end of exposure (mg/g creatinine)	0.18 ± 0.20	0.26 ± 0.12	0.21 ± 0.18	0.08/ns/ns
111TCE in blood at the end of exposure (µg/l)	362.97 ± 91.05	306.53 ± 152.27	371.13 ± 240.64	ns/ns/ns
Exhaled 111TCE at the end of exposure (ppm)	27.31 ± 11.09	28.28 ± 10.36	27.47 ± 6.93	ns/ns/ns

[*] Student's two-tailed t-test between men and women with hormonal contraceptive

[**] Student's two-tailed t-test between men and women without hormonal contraceptive

[***] Student's two-tailed t-test between women with and without hormonal contraceptive

with ns: not significant ($p > 0.1$)

3.4. Genotypes

Genotyping results for the CYP2E1*5 allele showed that among the participants, only one person presented the heterozygous type variant (c1/c2). Concerning the CYP2E1*6 allele, results showed that 21 volunteers (84 %) had the wild type variant (TT) and four (16 %) the heterozygous one (TA). Among the carriers of the mutant allele, one belonged to the group of men, another to the group of women with hormonal contraceptive and two to the group of women without hormonal contraceptive. For the latter, one of both was also the carrier of the heterozygous type variant (c1/c2) for genotype CYP2E1*5.

In view of the fact that the number of volunteers participating to the study is low, the number of persons carrying the mutant alleles of the CYP2E1 genotypes is even lower. Thus it was not possible to perform a statistical analysis on the genotype CYP2E1*5. Table 4 summarizes the results obtained after performing an ANCOVA analysis on the different biomarkers of exposure levels obtained for the studied volunteers groups.

Table 4. Results (F-ratios and p-values obtained from ANCOVA analysis) in order to identify potential confounders in evaluating differences, when exposed to organic solvents, between men and women with hormonal contraceptive (abbreviated in the table as horm. contr.), between men and women without hormonal contraceptive and between women with hormonal contraceptive and those without. Potential confounders considered: Body Mass Index (BMI), body fat (BF) and genotype CYP2E1*6.

Independent variables	**Dependent variables**									
	$C_{MEK,urine}$ (mg/l)		**$C_{free1M2P,urine}$ (mg/l)**		**$C_{total,1M2P,urine}$ (mg/l)**		**$C_{TCE,urine}$ (mg/ g creatinine)**		**$C_{TCA,urine}$ (mg/ g creatinine)**	
	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value	F-ratio	p-value
					Men vs women with hormonal contraception					
sex	4.61	0.05	0.83	0.38	0.99	0.34	0.25	0.62	0.79	0.39
BMI	1.63	0.22	0.21	0.65	2.94	0.11	0.02	0.88	0.10	0.76
BF	12.03	0.003*	4.06	0.06**	0.92	0.35	0.11	0.74	0.05	0.83
*CYP2E1*6*	6.11	0.03	0.19	0.67	1.82	0.19	0.00	0.98	1.72	0.21
					Men vs women without hormonal contraception					
sex	4.06	0.07	0.75	0.41	0.02	0.88	0.76	0.40	0.02	0.90
BMI	0.34	0.57	0.18	0.68	0.27	0.61	0.08	0.78	0.17	0.69
BF	0.24	0.64	0.26	0.62	0.09	0.77	0.05	0.83	0.25	0.63
*CYP2E1*6*	1.38	0.27	0.94	0.36	0.29	0.59	0.91	0.36	0.83	0.38
					Women with hormonal contraception vs women without					
horm. contr.	11.89	0.006	16.71	0.002	1.23	0.29	8.88	0.01	2.51	0.15
BMI	0.31	0.59	0.18	0.68	0.77	0.40	0.32	0.59	0.02	0.89
BF	1.25	0.29	15.11	0.003***	0.01	0.93	0.02	0.89	0.00	0.97
*CYP2E1*6*	0.41	0.54	6.99	0.02	1.81	0.21	0.01	0.93	0.61	0.45

* Pearson correlation matrix (correlation coefficient: -0.55, p = 0.01)
** Pearson correlation matrix (correlation coefficient: -0.58, p = 0.01)
*** Pearson correlation matrix (correlation coefficient: -0.35, p = 0.19)

The statistical analysis showed that body fat and genotype could be potential confounders when differences in urinary levels of biomarkers of exposure are observed between men and women.

When comparing men with women using hormonal contraceptives, body fat clearly influenced urinary concentrations for MEK and 1M2P. The Pearson correlation matrix confirmed a negative association in both cases. The genotype CYP2E1*6 when considered as covariate also showed a statistically significant p-value but the fact that only two persons were carrier of the mutant allele among the compared groups makes it difficult to deduce any affirmation. Urinary MEK concentrations for both volunteers in question were 0.93 mg/l and 0.75 mg/l, respectively. For the other two persons who belonged to the group of women without hormonal contraception, the measured urinary MEK concentrations were 0.73 mg/l and 1.35 mg/l, respectively, the latter one being the volunteer carrying also the heterozygous type variant (c1/c2) for genotype CYP2E1*5. Thus, when comparing these values with the mean urinary MEK levels obtained for each group of participants (see Table 3), there may be a tendency to have a higher CYP2E1 activity when the participant is carrier of the mutant allele of the genotype CYP2E1*6 only.

When comparing men with women without hormonal contraception, results showed no statistically significant p-value.

Finally, when comparing women using hormonal contraceptives with those who are not under hormonal contraception, results showed an effect due to the use of exogenous hormones for the three studied solvents. Moreover, in the case of 1M2P, body fat and genotype seem to be potential confounders. But the Pearson correlation matrix did not confirm an association between body fat and our dependent variable. For the CYP2E1*6 genotype, it is once again difficult to deduce something relevant due to the low number of participants carrying the mutant allele. The woman using hormonal contraceptives had a value of 3.05 mg/l of free 1M2P in urine. For the

two women without hormonal contraception, the urinary free 1M2P concentrations were 2.86 mg/l and 4.07 mg/l, respectively, the latter one being the volunteer carrying also the heterozygous type variant (c1/c2) for genotype CYP2E1*5. The only man carrying the genetic variant of CYP2E1*5 had a value of 3.11 mg/l of free 1M2P in urine. Here, when comparing these values with the mean urinary free 1M2P levels obtained for each group of participants (see Table 3), any tendency cannot be deduced.

3.5. Symptom questionnaire

The self-reported symptom questionnaire was distributed every 2 hours during exposure. The medical responsible saw the volunteers for possible symptoms before exposure, after exposure and in the morning of the following day.

Highest ratings were obtained for methyl ethyl ketone followed by 1-methoxy-2-propanol and the lowest one for 1,1,1-trichloroethane. Considering only the irritation symptoms (eyes, nose, throat), we can observe statistically significant differences between men and women, the latter ones giving higher ratings. The Mann-Whitney U test gave p values lower than 0.1 for discomfort in the eyes for exposure to MEK (after two and four hours) and to 1M2P (after two hours), for discomfort in the nose after six hours of exposure to 1M2P and to 111TCE, and for discomfort in the throat after four hours of exposure to 1M2P. The solvent smell was perceived by most of the volunteers at the beginning of the exposure. It seems that men got used more to the solvent odour than women who gave still a rather high score at the end of exposure ($p = 0.01$ after four hours of exposure to MEK and $p = 0.06$ after six hours of exposure to 1M2P). There were also higher ratings among women concerning headache, after four and six hours of exposure to MEK ($p = 0.04$ and $p = 0.06$, respectively). Women were more tired than men after six hours of exposure to

MEK ($p = 0.06$) and the opposite was observed after four hours of exposure to 1M2P ($p = 0.03$). Finally, women had a higher feeling of intoxication than men at the end of exposure of MEK and 1M2P ($p = 0.09$ in both cases).

4. Discussion

Our study suggests that not only physiological differences between men and women but also differences due to sex hormones levels can have an impact on urinary concentrations of several indicators of exposure. The measured values are of the same order of magnitude as data found in literature (Caperos et al., 1982; Devanthéry et al., 2002; Imbriani and Ghittori, 2004). When comparing to the biological exposure indices, we can estimate, by taking into account the exposure conditions, that we generally do not exceed them. In the case of TCA, values are very low, which is due to its long half-life time. Accumulation is possible during the week and thus, it is not an appropriate biomarker for reflecting a daily exposure.

Univariate analysis of variance, performed after logarithmic transformation of the independent variables and by adjusting for potential confounders (body mass index, body fat, genotype), showed the following main observations:

- a negative correlation between body fat and urinary MEK as well as urinary free 1M2P concentrations when comparing men with women using hormonal contraceptives;
- a difference due to the genotype CYP2E1*6, when exposed to methyl ethyl ketone, with a tendency to increase CYP2E1 activity when volunteers are carrier of the mutant allele;
- an effect among women due to the use of hormonal contraceptive on the urinary levels of several biomarkers of exposure, with an increase of more than 50 % in metabolites concentrations and a decrease of up to 50 % in unchanged substances concentrations,

suggesting an increase in their metabolism rate.

Body composition can influence the toxicokinetic behaviour of a substance (Sato et al., 1991). Men have generally higher lean body mass and women have higher body fat. The distribution of lipophilic compounds can especially be modified, as higher body fat increases the distribution volume for a lipophilic substance. Thus a decrease in the excretion rate of both the substance itself and its metabolites can be a consequence. Final urinary concentrations should also be lower in women than in men. But when looking at the measured values of the different biological indicators of exposure, we can see that mean values obtained for women can be higher than those obtained for men as it is the case when the unchanged substance is determined. Moreover, among women, there is an obvious difference between those under hormonal contraceptive and those not. Thus parameters other than the physiological ones could explain the differences observed between the volunteer groups.

According to several authors (Tanaka, 1998; Clewell et al, 2002; Parkinson et al., 2004), enzymatic activities of CYP1A2, CYP3A4, CYP2D6, and CYP2E1 are usually higher in males than in females, a tendency reflected by our results but which can not be confirmed as the volunteers' phenotypes have not been measured.

Concerning genetic polymorphism in CYP2E1, Prieto-Castello et al. (2010) observed a reduced activity of CYP2E1 in the presence of CYP2E1*5 and CYP2E1*6 variant alleles. Our results showed the opposite, which could be explained by the very low number of carriers of the variant allele or by an eventual high interindividual variability in CYP2E1 enzyme activity (Bebia et al., 2004).

A recent publication about gender differences in drug toxicity (Nicolson et al., 2010) supports the idea that hormonal interaction could play a key role. Actually, the effect due to hormonal

contraceptive was the most outstanding from the ANOVA analysis albeit the number of women for this comparison was low.

In a recent review of Kennedy (2008) about hormonal regulation of hepatic drug-metabolizing enzyme activity, it is has been shown that estrogens decrease hepatic microsomal activity and drug clearance in rodents. Evidence from clinical studies (Scandlyn et al., 2008) suggests that the CYP2E1 enzyme activity is lower in females than in men. The author states that CYP2E1 appears to be estrogen-regulated and that a reduced activity has been described during periods when estrogen levels remain elevated (pregnancy) or during changes in hormone levels (puberty, exogenous hormone administration).

In fact, combined oral contraceptives increase the estrogen and progesterone levels while decreasing the gonadotropins level (Kennedy, 2008). The increase in estrogen has as consequence an increase in the sex hormone-binding globulin (SHBG) level (Panzer et al., 2006), which has a high affinity and a low capacity for testosterone and estradiol (Pugeat et al., 2009). Albumin regulates the access of steroids to their receptors (Baker, 2002). The increase in SHGB level can enable steroids, bound with low affinity to albumin, to bind to SHGB. These variations in protein binding levels will have an impact on the balance between bound and unbound substances, and thus on hormonal activity. Xenoestrogens, as ethinylestradiol in hormonal contraceptive, bind to SHGB with low affinity, and are generally bounded to albumin.

One possible consequence of this imbalance in protein levels due to hormonal contraceptives could be a lower free estradiol level. Actually, free estradiol level is lower in women under hormonal contraceptive than women without hormonal contraceptive (Bjornerem et al., 2004). Thus, when combining Kennedy's hypothesis with our observations, we conclude that the use of hormonal contraceptives like the combined contraceptive pill may increase CYP2E1 activity.

Moreover, it seems to be more correct to say that the free estradiol level influences CYP2E1 activity instead of the estrogen level.

When comparing results obtained for free and total urinary 1M2P, we observed that an important part of the 1M2P is present in its conjugated form. Thus it is relevant to determine the total urinary 1M2P concentration when assessing human exposure to 1M2P. Besides, women on hormonal contraceptives appeared to excrete a higher fraction as conjugate than those not taking hormonal contraception. This may indicate that sex hormones levels also influence the enzyme activity of phase II reactions (Miners et al., 1983; Kennedy, 2008).

Creatinine correction is usually used in spot urine analysis and accounts for differences in urine flow and concentration. However, creatinine excretion depends on the muscle mass, which is higher in men than in women. In fact, when urinary TCE and TCA concentrations were adjusted to creatinine, sex differences became more pronounced.

Regarding the average symptom ratings obtained for each group of volunteers, we noticed that women rated irritating symptoms generally higher than men. It is interesting to see that even at half the threshold limit value, volunteers perceived irritating symptoms, solvent smell and headache. Fatigue belonged also to the high rated symptoms, but as it has been shown in a previous study (Ernstgård et al., 2002), there was in general no significant difference between volunteers exposed to clean air and those exposed to a solvent, making this symptom unspecific for solvent exposure.

As volunteers of this study belonged to a narrow range of age, the biological variability due to age was limited. However, due to the low number of participants, as it is often the case for studies with human volunteers, we observed an important interindividual variability among the participants groups, especially among women who did not take hormonal contraceptive. Despite

a severe volunteer profile, some participants groups were less homogenous than others. Several factors could thus contribute to the observed variability within a group, as for example body fat or the CYP2E1 activity itself (Bolt et al., 2003). But regarding women without hormonal contraceptives, this variability could also be related to the hormonal variation during the menstrual cycle, as they were exposed at any point of their cycle. The hormonal fluctuations are characterized by high estrogen levels during ovulation and high progesterone levels during the luteal phase. Nevertheless, it was possible to underline some statistically significant differences between the studied human volunteer groups.

In conclusion, our results suggest that there exist differences between men and women when exposed to organic solvents which could be explained not only by physiological differences but also by differences in CYP2E1 enzyme activity. For same exposure conditions, biological exposure indices can differ between men and women, which can lead to a misinterpretation of biomonitoring results. This aspect should have its place in the approaches for setting limits of occupational exposure as the existing recommended biological limits for chemical substances are most based on a direct correlation with the corresponding threshold limit values.

Acknowledgments The present work was supported by the National Research Fund (FNR) in Luxembourg, the French Agency of Environmental and Occupational Health Safety (Anses) and the Swiss Federal Office of Public Health (FOPH). We thank first above all the late Dr Pierre-Olivier Droz for his support and supervision of the project. We want to thank all the volunteers who participated in this study. We also express our thanks to Ms Christine Arnoux, Ms Patricia Stephan, Ms Monique Strebel, Ms Julia Bersier, Ms Mégane Volet, Ms Nadège Gavillet, M. Yann Randin and M. Jean-Noël Lepdor for their skillful technical work, to Dr Gilles Bieler, Dr Frédéric Regamey, Dr Bastien Chiarini, Dr Roland Yerly and Dr Bernd Zeilfelder for their medical assistance, and to M. Horacio Herrera, M. Pierre-Alain Porchet, Ms Christine Arnoux and Ms Nicole Charrière for their general support throughout the whole project. Finally, our thanks also go to the whole research group of Professor Gunnar Johanson from the Work Environment Toxicology (Institute of Environmental Medicine, Karolinska Institutet, Sweden) for their support and insightful discussions.

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3.4. Paper IV

Sex differences in urinary levels of several biological indicators of exposure: a simulation study using a general compartmental based toxicokinetic model.

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ABSTRACT

Toxicokinetic modeling is a useful tool to describe or predict the behavior of a chemical agent in the human or animal organism. A general model based on four compartments was developed in a previous study in order to quantify the effect of human variability on a wide range of biological exposure indices.

The aim of this study was to adapt this existing general toxicokinetic model to three organic solvents, which were methyl ethyl ketone, 1-methoxy-2-propanol and 1,1,1,-trichloroethane, and to take into account sex differences. We assessed in a previous human volunteer study the impact of sex on different biomarkers of exposure corresponding to the three organic solvents mentioned above. Results from that study suggested that not only physiological differences between men and women but also differences due to sex hormones levels could influence the toxicokinetics of the solvents. These experimental data were used to validate the toxicokinetic models developed in this study.

Our results showed that it was possible to use an existing general toxicokinetic model for other compounds. In fact, most of the simulation results showed good agreement with the experimental

data obtained for the studied solvents. Results pointed out that for same exposure conditions, men and women can show important differences in urinary levels of biological indicators of exposure. Moreover, when running the models by simulating industrial working conditions, these differences could even be more pronounced.

In conclusion, a general and simple toxicokinetic model, adapted for three well known organic solvents, allowed us to show that metabolic parameters can have an important impact on the urinary levels of the corresponding biomarkers. These observations give evidence of an interindividual variablity, an aspect that should have its place in the approaches for setting limits of occupational exposure.

Key words: Toxicokinetic modeling - Human biomonitoring - Sex differences – Organic solvents

INTRODUCTION

Toxicokinetic (TK) modeling is a useful tool to describe or predict the behavior of a chemical agent in the human or animal organism. Interest in it arose from the need to relate internal concentrations of active compounds with the external exposure conditions.[1] Data-based pharmacokinetic models were first developed in the 1920's and several disciplines like inhalation anesthesia, chemical engineering, toxicology or computer sciences, contributed to their maturation throughout the last decades.[2] Fiserova-Bergerova for example contributed intensively to the development of toxicokinetic models for inhaled organic solvents in humans.[3-5]

Depending on the substance, the complexity of the kinetics and the literature data available, two types of models can be used.[6] Classical toxicokinetic (TK) models, represented by compartments within which the chemical is assumed to be homogeneously distributed, are suitable when limited toxicological data is available. They are limited from a structural point of view but can be used to make generalizations. Physiologically-based toxicokinetic (PBTK) models, which rely on the anatomical and physiological structure of the body, are preferred when the purpose is to know the substance's concentration in different tissues or organs. They are more informative and usually allow for a better extrapolation processes.

Several authors investigated the quantification of biological variability through TK/PBTK modeling. Droz et al.[7], for example, developed a PBTK model for differing workers under variable industrial environments while Pierrehumbert et al.[8] tried to develop a general compartmental based TK model. The variability extent index and the main parameters affecting biological indicators were investigated by Truchon et al.[9] while the impact of environmental variability was quantified by Berthet et al.[10].

The influence of sex on toxicokinetics may involve female-male differences in physical constitution (body water space, muscle mass, body fat, and blood flow), physiology (menopause and menstruation cycle), hormones (contraceptive pill) as well as metabolizing enzymes (Löf et al., 1998).[11]

Clewell et al.[12] have described the contribution of sex (and age) to biological variability by developing a PBPK model to determine the tissue concentration as a function of time of some xenobiotics. This work represents an initial attempt to provide a predictive toxicokinetic framework and should for now be considered to represent reasonable expectations rather than a quantitative tool.

It is presently still very difficult to assess the variability associated with biological monitoring results. Although we can establish a list of contributing factors, as shown in a framework for sex differences by Gochfeld[13], their relative importance is unknown for different chemicals of occupational interest. It is therefore presently impossible to use such information to interpret biological variability and make safer quantitative decisions when using biological monitoring of exposure.

The aim of this study was to adapt an existing general toxicokinetic model to three organic solvents, which were methyl ethyl ketone (MEK), 1-methoxy-2-propanol (1M2P) and 1,1,1,-trichloroethane (111TCE), and to take into account sex differences. The influence of sex on different biological indicators had been evaluated in a human volunteer study[14] by exposing volunteers to the three organic solvents mentioned above. Experimental data from that study was used to validate the present models.

MATERIAL AND METHODS

Model description

Pierrehumbert et al.[8] have developed a general compartmental model in order to quantify the effect of human variability on a wide range of biological exposure indices (BEIs). By applying it to four chemicals (toluene, phenol, lead and mercury), they showed its potential to be used for further substances. We actually underline this potential of the model by adapting it to three other compounds which are MEK, 1M2P and 111TCE.

The general compartmentally based toxicokinetic model developed by Pierrehumbert et al.(8) consists of four compartments and takes into account only absorption by inhalation, as BEIs establishment does not consider oral or skin absorption. In fact most of BEIs are based on a direct correlation with the threshold limit values (TLVs) which indicate the potential exposure by

inhalation of a worker. The air absorption rate is equal to the product of the exposure concentration and the alveolar ventilation scaled to body weight, the whole multiplied by the pulmonary retention if applied. The chemical is distributed between the central compartment and the peripheral one, or storage compartment. These compartments can be illustrated by different tissues, depending on the chemical. The distribution can be either flow or diffusion limited and includes the permeability and affinity of the tissue for the chemical. For transfer between central and peripheral compartments, rates are expressed as a function of blood flow, affinity and permeability. The metabolism, described with Michaelis–Menten kinetics, can give one or more metabolites, and can occur by serial or parallel metabolism. Elimination is represented by excretion in expired air, feces and urine, or by metabolism. The air excretion rate is equal to the product of the concentration in the central compartment and the alveolar ventilation scaled to body weight, the whole divided by the blood/air partition coefficient. For excretion, estimated rates are based either on the half-life of the chemical/metabolite, or on its clearance (e.g. bile flow for feces, urine flow for urine, alveolar ventilation for expired air) and volume of distribution. More detail about the mathematical equations describing the general model can be found in Pierrehumbert et al.[8].

This general model was adapted to MEK, 1M2P and 111TCE using toxicokinetic data available in the literature.[15-21] The simulation software used for the development of the TK models was Berkeley Madonna (version 8.0.1.), developed by Robert Macey and George Oster of the University of California at Berkeley (USA).

Human volunteer study

Experimental data were taken from a previous human volunteer study.[14] Three chemicals have been selected for their relatively low toxicity as well as their metabolic and physiological

properties: MEK, watersoluble and highly metabolized, 1M2P, amphiphile and highly metabolized, and 111TCE, liposoluble and poorly metabolized.

Controlled human exposures were carried out in a 12 m^3 exposure chamber for each solvent separately, during six hours and at half of the threshold limit value (TLV). The human volunteers groups were composed of ten young men and fifteen young women. The latter were separated into two sub-groups by taking into account the use or not of hormonal contraceptive. The characteristics of each volunteer group are summarized in Table I (Tomicic et al.[14]).

TABLE I. Characteristics of each volunteer group. Arithmetic mean values (± SD: standard deviation) are indicated; n = number of volunteers.

	Men (n=10)	**Women (n=15)**	***Women with hormonal contraceptive (n=10)***	***Women without hormonal contraceptive (n=5)***
Age (years)	22.4 ± 2.2	23.2 ± 2.9	22.6 ± 2.1	24.4 ± 4.2
Body weight (kg)	70.7 ± 10.3	61.9 ± 8.7	60.1 ± 7.2	65.6 ± 11.2
Body height (cm)	177.5 ± 3.2	165.8 ± 3.7	167 ± 3.8	163.4 ± 2.3
Body Mass Index (-)	22.3 ± 2.6	22.5 ± 3.2	21.6 ± 2.6	24.5 ± 3.7
Body fat (% of body weight)	11.3 ± 5.2	22.1 ± 6.9	19.8 ± 4.6	26.6 ± 8.9

Following biological indicators of exposure were determined: urinary MEK, urinary 1M2P (conjugated and total), 111TCE in blood and in expired air, metabolites of 111TCE (trichloroethanol (TCE) and trichloroacetic acid (TCA)) in urine. The corresponding BEIs are summarized in Table II (Tomicic et al.[14]).

TABLE II. Summary of the selected organic solvents and their corresponding urinary biomarkers of exposure, with the existing occupational exposure limit values and biological exposure indices for different countries (USA, Germany, Switzerland)

Biological determinant	**TLV*/MAK**/VME*** (ppm)**	**BEI*/BAT**/VBT*** in urine (mg/l)**
Methyl ethyl ketone (MEK)	200/200/200	2/5/5 [a]
1-Methoxy-2-propanol (1M2P)	100/100/100	-/15/20 [a]
1,1,1-Trichloroethane (111TCE)	350/200/200	-
Trichloroethanol (TCE)		30/-/- [a,b]
Trichloroacetic acid (TCA)		10/-/- [b]

* The American Conference of Industrial Hygienists (ACGIH) sets threshold limit values (TLV) and biological exposure indices (BEI).

** The German Research Foundation (Deutsche Forschungsgemeinschaft - DFG) sets "Maximale Arbeitsplatz-Konzentrationen" (MAK) and "Biologische Arbeitsstoff-Toleranzen" (BAT).

*** The Swiss National Accident Insurance Fund (Schweizerische Unfallversicherungsanstalt - Caisse nationale suisse d'assurance en cas d'accidents - Istituto nazionale svizzero di assicurazione contro gli infortuni - SUVA) sets "valeurs (limites) moyennes d'exposition" (VME) and "valeurs biologiques tolérables" (VBT).

[a] ES: end of shift [b] EW: end of week [c] PS: prior to shift

Model parameters

The only measured parameters in the frame of the human volunteer study were body weight, body height and body fat. Thus their values in the models corresponded to the arithmetic means obtained for each quite homogenous group of human volunteers who have participated to this study. Values for general physiological parameters like alveolar ventilation, cardiac output or organ blood flows corresponded to the values of a reference man. These were scaled in function of body weight. Otherwise, values were taken from literature, like partition coefficients or metabolic parameters.

Simulation conditions

The three toxicokinetic models were first calibrated with the experimental data obtained during the previous human volunteer study[14].

In a second step, simulations were performed to mimic an occupational exposure at the TLV (8 h per day, 5 days per week, physical activity of 50 W for 12 h per day and at rest for the resting 12 hours).

Lastly, concentrations of the studied substances can be predicted in other biological matrices or tissues, as for example in blood (corresponding to the central compartment of the model) or in fat (corresponding to the peripheral compartment of the model).

RESULTS

Toxicokinetic models for MEK, 1M2P and 111TCE

In most occupational exposure settings, the primary intake route for the three studied solvents is inhalation. The different compartmental TK models for the studied solvents, all metabolized via the cytochrome P450 mixed-function oxidase system, are illustrated on Figure 1.

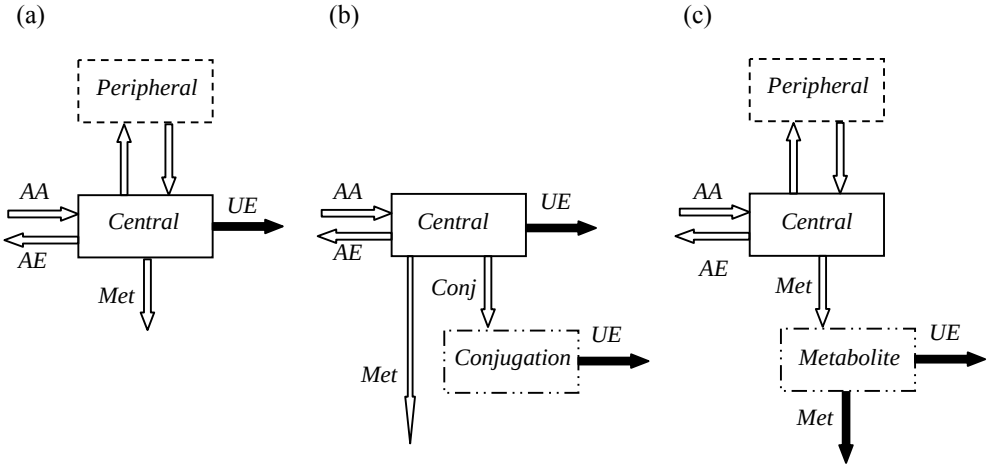


FIGURE 1. Illustration of the different compartmental based TK models for (a) MEK, (b) 1M2P and (c) 111TCE, with the black arrows indicating the biological determinants considered in this study, and with following flow rates air absorption (AA), air excretion (AE), urinary excretion (UE), metabolism (Met) and conjugation (Conj) expressed as [mg/h].

MEK is metabolized to 2,3-butanediol, 2-butanol and 3-hydroxy-2-butanone (major metabolite), all excreted in urine. A low fraction of unchanged solvent is excreted in the exhaled air. The urinary concentration of MEK at the end of the shift has been recommended as the most appropriate biological exposure indicator. MEK is nearly equally distributed between water and fat containing tissues.[22] Thus the model is composed by a central compartment representing the total body water (TBW) and a peripheral compartment for the fatty tissues.

1M2P belongs to the family of the propylene glycol ethers (PGE) which exists under the form of two isomers, alpha-isomer and beta-isomer. The latter one, considered as an impurity in commercial use, is first transformed into 2-alkoxy acetaldehydes by alcohol dehydrogenases, and then into alkoxyacetic acids by aldehyde dehydrogenases, a toxic metabolite. Alpha-PGE are primarily metabolized to propylene glycol, carbon dioxide and glucuronide as well as sulfate conjugates of the parent compound. The urinary 1M2P concentration at the end of the shift is the appropriate biomarker. A central compartment equivalent to TBW and a second compartment illustrating the conjugation of 1M2P constitute the TK model for 1M2P.

111TCE is metabolized to TCE and TCA, both excreted in urine. Other minor metabolites (carbon dioxide, acetylene) are excreted in the exhaled air. The corresponding biological exposure indices are the urinary concentration of both metabolites and the blood concentration of the parent compound. The 111TCE concentration in the exhaled air can also be considered as a biomarker of exposure. As the exposure scenarios were carried out during one day, urinary TCE is the most appropriate biomarker for the model calibration with the experimental data. Thus the TK model includes a central compartment equivalent to TBW, a peripheral one constituting the fatty tissues because of its high liposolubility, and a third compartment for the considered metabolite.

The general physiological parameters used in the simulations are presented in Table III whereas the chemical specific parameters for each TK model are represented in Table IV.

TABLE III. General physiological parameters used for the TK models

Parameters	**Symbol**	**At rest/50 W**
Body weight [kg]	BW	Arithmetic mean value for each group of human volunteers
Body height [cm]	BH	Arithmetic mean value for each group of human volunteers
Body fat [kg]	BF	Arithmetic mean value for each group of human volunteers
Lean body mass [kg]	LBM	LBM = BW – BF[a]
Total body water [kg]	TBW	For a man : -12.86 + 0.1757 * BH + 0.3331 * BW [a] For a woman : -2.097 + 0.1069 * BH + 0.2466 * BW[a]
Cardiac output [l/(h*kg$^{0.7}$)]	Q_c	18.0[b]/30.8[b]
Alveolar ventilation [l/(h*kg$^{0.7}$)]	V_{alv}	18.0[b]/2.1951*Q_c[b]
Urinary excretion rate [ml/(h*kg$^{0.82}$)]	k_{ur}	1.848[c]
Creatinine excretion rate [µmol/(h*kg$^{0.9}$)]	k_{cr}	12.06[c]

[a] Fiserova-Bergerova[23]
[b] Thomas et al.[24]
[c] Laparé et al.[25]

TABLE IV. Chemical specific parameters used for the TK models

Parameters	**Symbol**	**At rest/50 W**
MEK		
Threshold limit value [mg/m^3]	TLV	590
Molecular weight [g/mol]	MW	72
Exposure concentration [mg/m^3]	C_{exp}	282.9
Pulmonary retention [-]	R_{pulm}	0.558[a]
Volume of the central compartment expressed as a fraction of BW [-]	FV_c	1*TBW/BW
Volume of the peripheral compartment expressed as a fraction of BW [-]	FV_p	1*BF/BW
Fraction of cardiac output in the peripheral compartment [-]	BF_p	0.06[b]/0.05[b]
Fraction of cardiac output in metabolism [-]	BF_1	0.26[b]/0.16[b]
Blood/air partition coefficient [-]	P_{blood_air}	125[a]
Central/blood partition coefficient [-]	P_{c_air}	0.856[a]
Peripheral/central partition coefficient [-]	P_{p_c}	1.296[a]
Metabolism		
Michaelis-Menten maximum rate** [mg/(h*kg$^{0.75}$)]	VM_1	5.44[c]
Michaelis-Menten constant* [mg/l]	KM_1	0.63[c]
1M2P		
Threshold limit value [mg/m^3]	TLV	360
Molecular weight [g/mol]	MW	90.12
Exposure concentration [mg/m^3]	C_{exp}	177.1
Pulmonary retention [-]	R_{pulm}	0.9[d]
Volume of the central compartment expressed as a fraction of BW [-]	FV_c	1*TBW/BW
Fraction of cardiac output in metabolism [-]	BF_1	0.26[b]/0.16[b]
Blood/air partition coefficient [-]	P_{blood_air}	12383[e]
Central/blood partition coefficient [-]	P_{c_blood}	12280[e]
Metabolism		
Michaelis-Menten maximum rate*,** [mg/(h*kg$^{0.75}$)]	VM_1	22[f]
Michaelis-Menten constant* [mg/l]	KM_1	45[f]
Conjugation		
Michaelis-Menten maximum rate*** [mg/(h*kg$^{0.75}$)]	VM_2	0.2[f]
Michaelis-Menten constant* [mg/l]	KM_2	80[f]
Urinary excretion rate for conjugated 1M2P [h^{-1}]	k_{conj}	0.2[d]
111TCE		
Threshold limit value [mg/m^3]	TLV	1080
Molecular weight [g/mol]	MW	133.40
Molecular weight of metabolite [g/mol]	MW_1	149.40
Molecular weight of creatinine [g/mol]	MW_{cr}	113.12

Exposure concentration [mg/l]	C_{exp}	545.6
Pulmonary retention [-]	R_{pulm}	0.25[g]
Volume of the central compartment expressed as a fraction of BW [-]	FV_c	1*TBW/BW
Volume of the peripheral compartment expressed as a fraction of BW [-]	FV_p	1*BF/BW
Volume of the metabolite compartment expressed as a fraction of BW [-]	FV_1	0.026[h]
Fraction of cardiac output in peripheral compartment [-]	BF_p	0.06[b]/0.05[b]
Fraction of cardiac output in metabolite compartment [-]	BF_1	0.26[b]/0.16[b]
Blood/air partition coefficient [-]	P_{blood_air}	2.53[i]
Central/air partition coefficient [-]	P_{c_air}	2.53[i]
Peripheral/air partition coefficient [-]	P_{p_air}	263[i]
Metabolite/air partition coefficient [-]	P_{M1_air}	8.6[i]
Metabolism		
Michaelis-Menten maximum rate[**] [mg/(h*kg$^{0.75}$)]	VM_1	0.42[i]
Michaelis-Menten constant [mg/l]	KM_1	5.75[i]
Metabolic rate TCE $\rightarrow$ TCA [1/(h*kg$^{-0.3}$)]	k_{M1_M2}	0.069[j]
Excretion		
TCE urinary excretion rate [1/(h*kg$^{-0.3}$)]	k_{u1}	0.093[j]

[a] Liira et al.[22]
[b] Thomas et al.[24]
[c] Thrall et al.[19]
[d] Devanthéry[26]
[e] Johanson et al.[27]
[f] Corley et al.[20]
[g] Nolan et al.[28]
[h] Tardif et al.[29]
[i] Reitz et al.[15]
[j] Caperos et al.[30]

[*] When fitting toxicokinetic models to our experimental data obtained for men, metabolic parameters have been adapted, with

- KM_1 = 1.1 mg/l for the MEK model ;
- VM_1 = 30 mg/(h*kg$^{0.75}$), KM_1 = 30 mg/l and KM_2 = 95 mg/l for the 1M2P model.

[**] When fitting toxicokinetic models to our experimental data obtained for women without hormonal contraceptive, metabolic parameters have been modified, by reducing the Michaelis-Menten maximum rate value by half, with

- VM_1 = 2.7 mg/(h*kg$^{0.75}$) for the MEK model ;
- VM_1 = 15 mg/(h*kg$^{0.75}$) and VM_2 = 0.1 mg/(h*kg$^{0.75}$) for the 1M2P model ;
- VM_1 = 0.21 mg/(h*kg$^{0.75}$) for the 111TCE model.

[***] Based on Miners et al.[31] observations, the Michaelis-Menten maximum rate value for conjugation was reduced by 45 % for the group of women without hormonal contraceptive, with

- VM_2 = 0.11 mg/(h*kg$^{0.75}$) for the 1M2P model.

Experimental data *vs* simulated data

The conclusion of the human volunteer study[14] was that there exist differences between men and women when exposed to organic solvents which could be explained not only by physiological differences but also by differences in CYP2E1 enzyme activity. An analysis of variance mainly showed an effect on the urinary levels of several biomarkers among women due to the use of hormonal contraceptive, with an increase of about 50 % in the metabolism rate.

In a first step, the TK models of the three studied solvents were calibrated with the experimental data obtained for the male human volunteers, showing generally good agreement between both. Metabolic parameters for MEK and 1M2P have been fitted to our experimental data, with focusing on a good agreement for the urinary levels measured during the exposure to the solvent, that means for t=2, t=4 and t=6 hours. In literature, these values were actually fitted to data[19,20], for MEK and 1M2P but not for 111TCE.

Thus, in the case of MEK metabolism, the Michaelis-Menten constant value taken from literature was adapted to our experimental data, with KM_1 = 1.1 mg/l. In the case of 1M2P metabolism, the Michaelis-Menten maximum rate and constant values taken from literature were adapted to our experimental, with VM_1 = 30 mg/(h*$kg^{0.75}$), KM_1 = 30 mg/l and KM_2 = 95 mg/l.

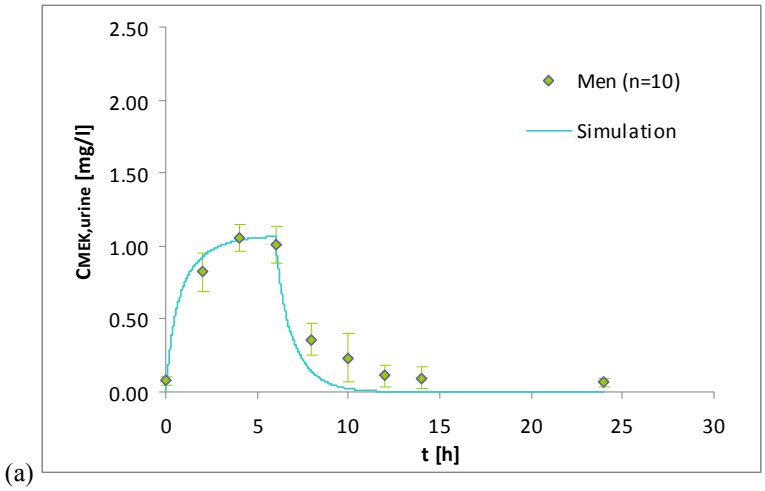
When changing physiological parameters with the values for the female human volunteers groups, biological indicators levels were slightly influenced but still in good agreement with the experimental data.

The main observation was that the agreement between experimental and simulated values was merest for the group of women without hormonal contraceptive. On basis of the emitted hypothesis in the previous human volunteer study[14], suggesting that there was a mean increase of about 50 % in the metabolism rate due to contraceptive hormones, metabolic parameters for the group of women without hormonal contraceptive were modified, by reducing the Michaelis-

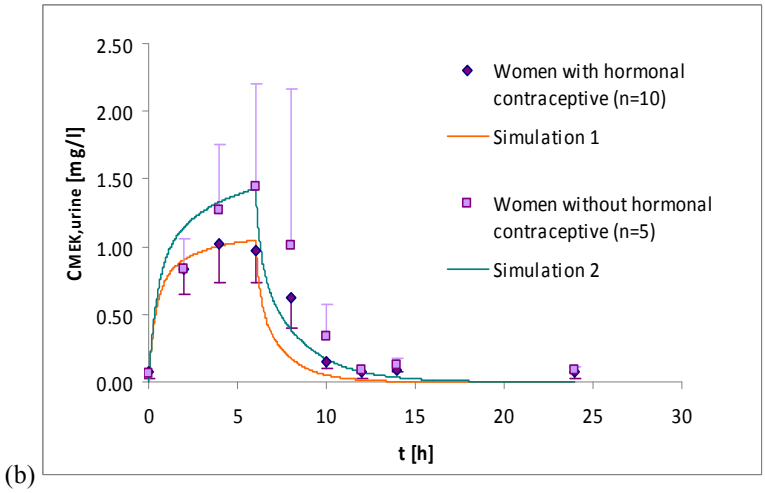
Menten maximum rate value by half. Thus, for the MEK model, VM_1 = 2.7 mg/(h*$kg^{0.75}$), for the 1M2P model, VM_1 = 15 mg/(h*$kg^{0.75}$), and for the 111TCE model, VM_1 = 0.21 mg/(h*$kg^{0.75}$).

Concerning phase II metabolism, Miners et al.[31] showed that glucuronidation was induced in women using oral contraceptives. In the previous human volunteer study[14], women on hormonal contraceptives appeared to excrete a higher fraction as conjugate than those not taking hormonal contraception, indicating also that sex hormones levels may influence the enzyme activity of phase II reactions but differences were not statistically significant due to a high variability. Based on Miners et al.[31] observations, the Michaelis-Menten maximum rate value for conjugation was reduced by 45 % for the group of women without hormonal contraceptive. Thus, for the 1M2P model, VM_2 = 0.11 mg/(h*$kg^{0.75}$).

Figures 2 to 5 illustrate the comparison of experimental data with simulated data for the different urinary indicators of exposure considered.



(a)



(b)

FIGURE 2. Comparison of experimental data with simulated data for urinary MEK obtained (a) in men and (b) in women. Urinary MEK concentrations as function of time were obtained after 6 hours of exposure to 99.15 (± 5.29) ppm MEK. Arithmetic mean values (± SD: standard deviation) are indicated; n = number of volunteers.

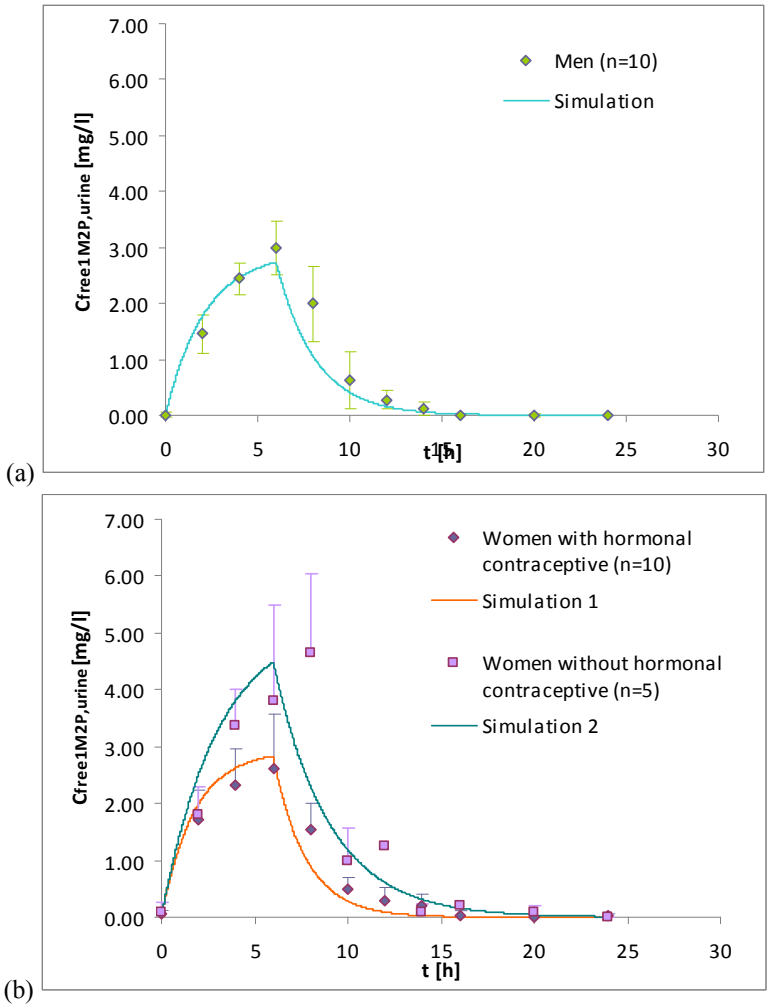


FIGURE 3. Comparison of experimental data with simulated data for urinary free 1M2P obtained (a) in men and (b) in women. Urinary free 1M2P concentrations as function of time were obtained after 6 hours of exposure to 53.22 (± 3.04) ppm of 1M2P. Arithmetic mean values (± SD: standard deviation) are indicated; n = number of volunteers.

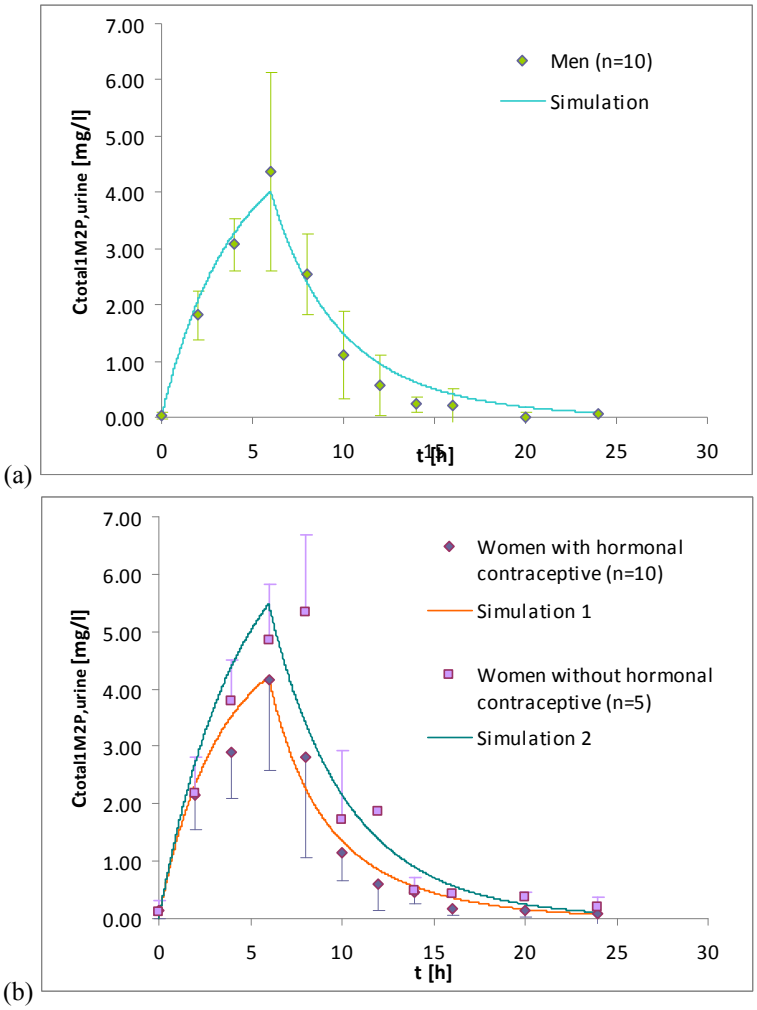


FIGURE 4. Comparison of experimental data with simulated data for urinary total 1M2P obtained (a) in men and (b) in women. Urinary total 1M2P concentrations as function of time were obtained after 6 hours of exposure to 53.22 (± 3.04) ppm of 1M2P. Arithmetic mean values (± SD: standard deviation) are indicated; n = number of volunteers.

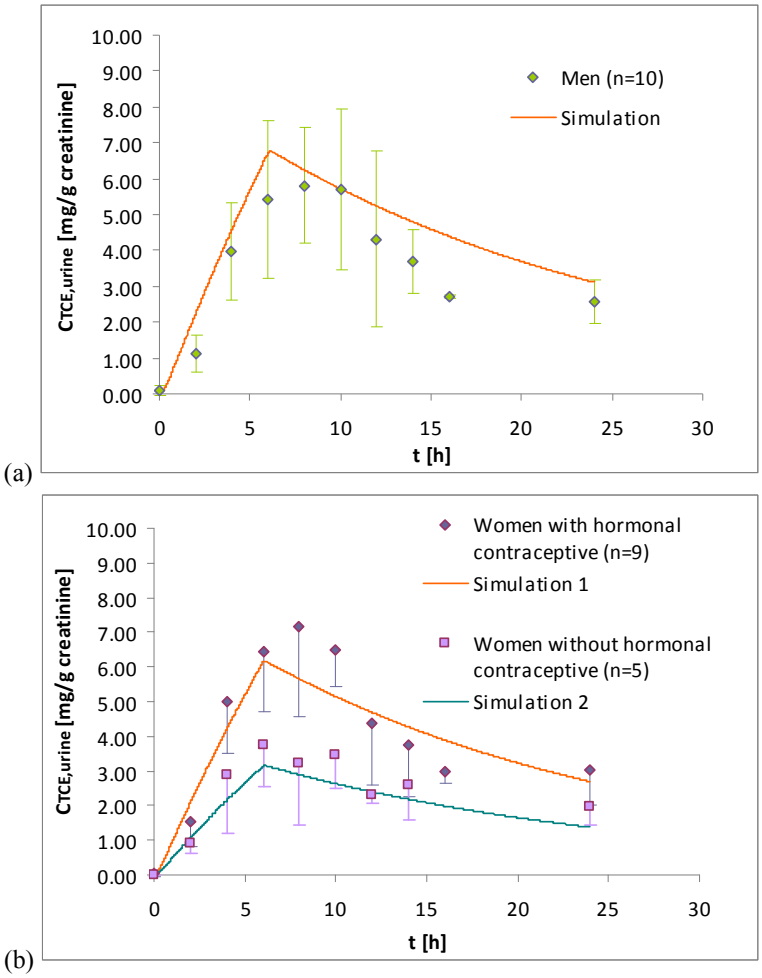


FIGURE 5. Comparison of experimental data with simulated data for urinary TCE obtained (a) in men and (b) in women. Urinary TCE concentrations as function of time were obtained after 6 hours of exposure to 102.55 (± 3.19) ppm of 111TCE. Arithmetic mean values (± SD: standard deviation) are indicated; n = number of volunteers.

Table V summarizes the experimental data obtained at the end of exposure in the human volunteer study[14] on one hand and the simulated values obtained at the end of exposure within the TK models on the other hand, for the considered biological determinants.

TABLE V. Summary of the different biomarkers values (experimental and simulated) obtained at the end of exposure. Arithmetic mean values (± SD: standard deviation) are indicated for experimental data.

	Biological indicators levels at the end of exposure			
	$C_{MEK,urine}$ [mg/l]	**$C_{free1M2P,urine}$ [mg/l]**	**$C_{total1M2P,urine}$ [mg/l]**	**$C_{TCE,urine}$ [mg/g creatinine]**
Men				
Experiment	1.00 ± 0.13	2.99 ± 0.49	4.36 ± 1.76	5.42 ± 2.19
Simulation	1.07	2.72	4.06	6.76
Women with hormonal contraceptive				
Experiment	0.97 ± 0.23	2.61 ± 0.96	4.17 ± 1.58	6.46 ± 1.73
Simulation	1.04	2.81	4.21	6.31
Women without hormonal contraceptive				
Experiment	1.44 ± 0.76	3.81 ± 1.68	4.85 ± 0.97	3.77 ± 1.24
Simulation (same metabolic parameters)	1.03	2.83	4.21	5.76
Simulation (modified metabolic parameters)	1.42	4.46	5.47	2.99

Finally, the goodness-of-fit between experimental data and simulated data can be assessed by linear regression analysis assuming that the slope yields the value of 1 in the case of perfect agreement. Thus a scatterplot of the predicted data *versus* the observed ones (see Figure 6) suggests that the toxicokinetic models seem adequate for the three studied substances, at least for the values obtained during exposure. During the elimination phase, the models show less agreement, especially for MEK and free 1M2P in urine.

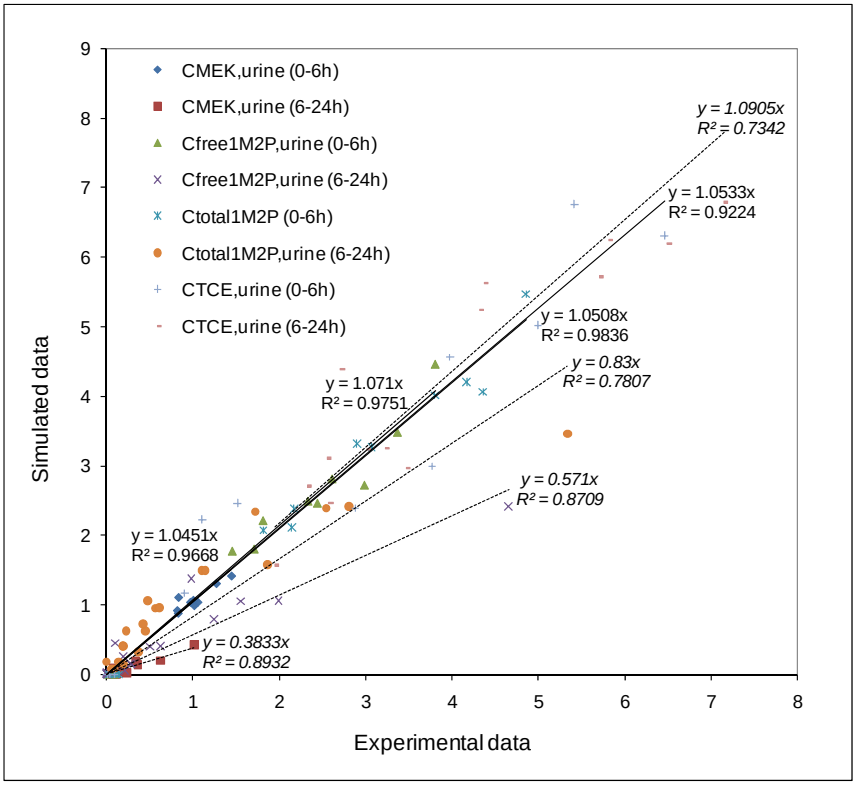
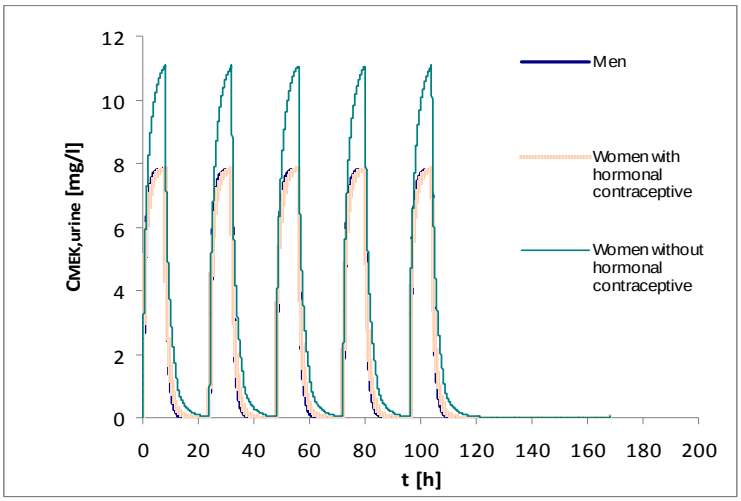
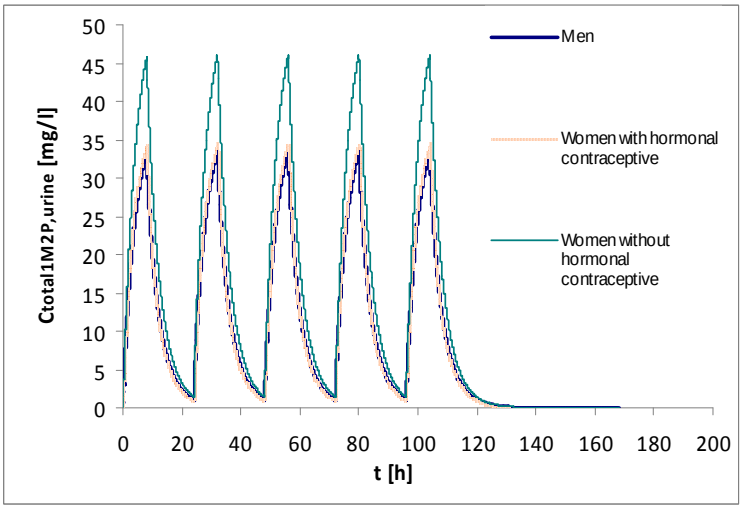


FIGURE 6. Predicted data versus experimental data for the studied urinary biomarkers of exposure. Linear regression has been done for data obtained during exposure to the solvent (0 to 6 hours) on one hand and for data obtained during the elimination phase (6 to 24 hours) on the other hand.

In a second step, simulations were done for estimating the urinary levels of the different biological biomarkers when workers were exposed during 8 hours per day, 5 days per week, at a 50 W work load, to the threshold limit value of each solvent studied. Predictions are illustrated in Figure 7 for each solvent and for each studied human volunteer group.



(a)



(b)

(c)

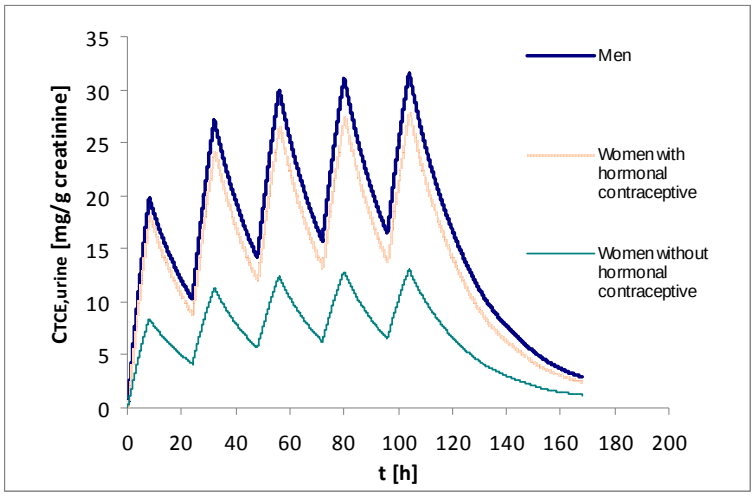


FIGURE 7. Simulation of mean urinary levels of biological indicators of exposure in workers (by considering men, women with and without hormonal contraceptive) exposed during 8 hours per day, 5 days per week, at a 50 W work load, to the threshold limit value of (a) MEK, (b) 1M2P and (c) 111TCE

From these models, it is also possible to get predictions for the concentrations of the studied substances in other biological matrices or tissues, as for example in blood (corresponding to the central compartment of the model) or in fat (corresponding to the peripheral compartment of the model).

When taking the same simulation conditions as just before, the model predicted a maximal blood concentration of 7.8 mg/l for MEK and of 21.1 mg/l for total 1M2P in men as in women with hormonal contraceptives. In women without hormonal contraceptives, the predicted levels corresponded to 11.1 mg/l for MEK and 34.5 mg/l for total 1M2P. As for the urinary biomarkers of exposure, blood levels are about 50 % higher in women without hormonal contraceptives. In the previous human volunteer study[14], blood levels for MEK and 1M2P have not been measured and thus, there are no experimental data for confirming the observed differences among the predicted values.

In the case of 111TCE, the model predicted, after 8 hours of exposure, a maximal blood concentration of 0.62 mg/l in each group of volunteer (the value is equal to 0.28 mg/l when running the model under the same exposure conditions as in the human volunteer study), showing no differences between them. This observation can in fact be confirmed by the experimental data obtained in the previous human volunteer study.[14] End-exposure values obtained for men, women with and without hormonal contraceptive, respectively were 362.97 (± 91.05), 306.53 (± 152.27) and 371.13 (± 240.64) µg/l, with no statistically significant differences between the different groups.

111TCE levels predicted in fat (see Figure 8) were equal to 0.13 mg/l in men, 0.07 mg/l in women with hormonal contraceptives and 0.06 mg/l in those without. Here the observed differences were of course related to the differences in body fat among the different groups of volunteers, indicating that women have a two-fold higher body fat value than men.

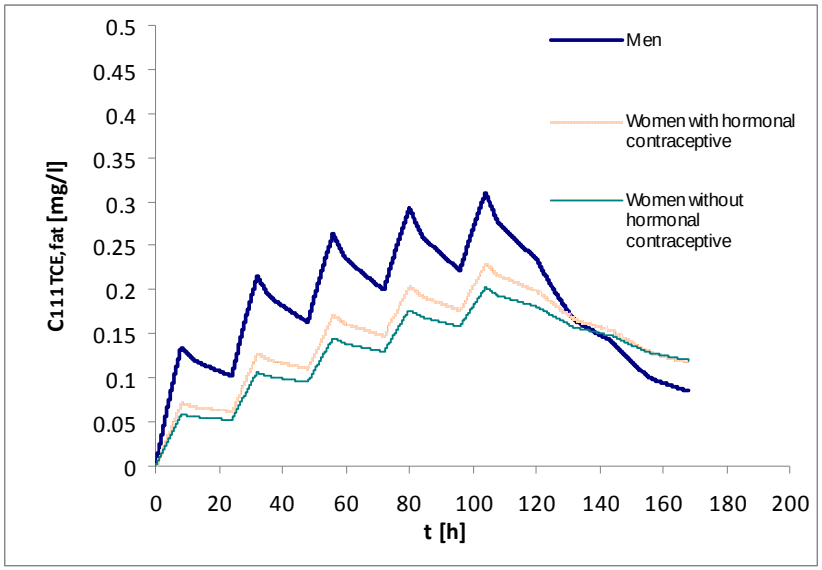


FIGURE 8. Simulation of mean 111TCE fat levels in workers (by considering men, women with and without hormonal contraceptive) exposed during 8 hours per day, 5 days per week, at a 50 W work load, to the threshold limit value of 111TCE

DISCUSSION

Our results showed that it was possible to use an existing generic toxicokinetic model for other compounds by adapting it to every studied organic solvent. In fact, most of the simulation results showed good agreement with the experimental data obtained.

When looking at the measured urinary MEK concentrations obtained by Liira et al.[17] and when taking into account the fact that MEK kinetics are dose-dependent,[18] our experimental data match those within the literature. Devanthéry et al.[32] measured urinary 1M2P concentrations in human volunteers at an exposure concentration, amongst others, 50 ppm, which were of the same order of magnitude as the experimental data of this study. The relevant literature available for 111TCE is abundant. As a matter of fact, Lu et al.[33] found fifteen published PBPK models in rats and humans, and among the eleven studies selected for experimental data, four were studied in human volunteers. The more relevant study, performed in similar exposure conditions, was a human volunteer study carried out by Monster et al.[34]. The measured urinary excretions of the 111TCE's metabolites found in this study were similar to our results.

When simulations were performed with the same values for the metabolic parameters but with the data measured for the female human volunteer groups, results were quite similar to those obtained for men, indicating that physiological parameters as body weight, body height and body fat, have a rather weak influence on the urinary levels of the different biological indicators of exposure. Women with hormonal contraceptive showed values close to the ones determined for men, which was not the case for women without hormonal contraceptive.

Thus, metabolic parameters were modified for the latter ones by reducing the Michaelis-Menten maximum rate value by half for each solvent, with the assumption that the differences observed between the human volunteers groups are due to an effect on the CYP2E1 activity by

exogenous hormones[14], which suggested that there was a mean increase of about 50 % in the metabolism rate due to contraceptive hormones. Simulations with the modified metabolic parameters values resulted in a better agreement with the experimental data obtained for women without hormonal contraceptive. Above all, these simulations showed that metabolic parameters had much larger effects than the physiological parameters on the studied biological determinants.

Physiological parameters are expressed in function of body weight and thus take into account possible male/female differences in pulmonary ventilation, cardiac output and organ blood flows, generally due to body size. Moreover the fact that differences are observed between women underlines Gochfeld's hypothesis[13] that toxicokinetic differences mainly involve metabolism. The author also stated that there is still a tendency to believe that most sex differences relate to morphology and body size. He pointed out that the differences in susceptibility between men and women need to be incorporated in risk assessments, as most toxicological studies still focus on only one sex.

The predictive simulations done over a week within working conditions indicated that exposure for women without hormonal contraceptive can be overestimated in the case of MEK and 1M2P but underestimated in the case of 111TCE. For the latter, Truchon et al.[9] have estimated the extent of the variability for its biomarkers, indicating that TCE urinary levels can vary from 11 to 80 mg/l due to biological variability, without taking into account differences due sex and age. Urinary TCE values in this study were adjusted by creatinine to correct for variable dilutions among spot urine samples. Results would be lower when expressed as mg/l. Thus, in this case, sex differences could contribute to enlarge the extent of the variability for the considered biological indicator.

Moreover, the urinary levels in biomarkers predicted with our TK models were compared to the existing BEIs. Interestingly, urinary levels following TLV exposures to MEK and 1M2P

were, in a general way, found to be higher than the current BEI, suggesting that work load can have a significant impact on biological exposure indicators.[35]

The main limitation of the models concerns the elimination phase of the urinary biomarkers of exposure, mainly for urinary MEK and urinary 1M2P (free and total). In fact agreement between observed and predicted values is weaker and this can lead to an over- or underestimation of the corresponding exposure level. In the case of MEK, an explanation could be the metabolic saturation, as in humans it begins at relatively low levels of exposure[18], and greater amounts of MEK would be expected to be excreted via the kidney. For 1M2P, the high variability in glucuronidation observed in the previous human volunteer study[14] could explain the differences observed between experimental and simulated data.

But a predictive model should be used as a further tool in exposure assessment. BEIs are guidelines established when a worker is exposed during a whole workshift to the TLV value of the considered chemical. When comparing with guidelines, the most important is to know about biomarkers levels at the end of exposure. Thus sampling time of a biological fluid plays a crucial role when evaluating a worker's exposure to a chemical. From this point, the exposure level can be extrapolated by simulation. Then an average value of the exposure level over the whole workshift should be compared with the corresponding threshold limit value.

In conclusion, a general and simple toxicokinetic model, adapted for three well known organic solvents, allowed us to show that metabolic parameters can have an important impact on the urinary levels of the corresponding biomarkers. Indeed, experimental data pointed out that for same exposure conditions, men and women can show a difference of 50 % among the urinary biological indicators levels. Moreover, when running the models by simulating industrial working conditions, these differences could be even more pronounced. These observations give

evidence of an interindividual variability, an aspect that should have its place in the approaches for setting limits of occupational exposure.

ACKNOWLEDGMENTS

The present work was supported by the National Research Fund (FNR) in Luxembourg, the French Agency of Environmental and Occupational Health Safety (Anses) and the Swiss Federal Office of Public Health (FOPH). We thank first above all the late Dr Pierre-Olivier Droz for his support and supervision of the project. We express our thanks to the group of Professor Gunnar Johanson from the Karolinska Institute (Stockholm, Sweden), particularly Ms Anna-Karin Mörk, for their support. We also want to thank Professor Thierry Buclin from the Division of Clinical Pharmacology and Toxicology (University Hospital, Lausanne, Switzerland) for the insightful discussions.

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4 General discussion

The purpose of this study was to evaluate individual differences in chemical disposition due to age and sex. The results of this study suggest that age and sex can have an impact on urinary levels of biological indicators of exposure, which can be explained by differences in the toxicokinetics of the chemical agents. Overall literature review on toxicokinetic differences due to age and sex showed a list of parameters that may contribute to a modified response of the organism when exposed to a substance. Some were extensively described, like physiological data for example, while others were inconsistent, which was the case for metabolic parameters, or presented gaps.

Thomson et al. (2009) carried out a thorough literature review about physiological data for healthy and health-impaired elderly. They concluded that the overall data gap across the age groups concerned particularly tissue and blood composition data. Moreover physiological measurements in health-impaired older adults were particularly limited. For metabolic functions, they suggested that the lack of such data is not crucial since the decline in metabolic activity in elderly persons might be more a consequence of the decrease in liver blood flow and weight than a change in enzyme activity which was also assumed in the first part of this project. The tentative description of age differences in biological monitoring of exposure using existing TK models, developed by Pierrehumbert et al. (2002) for a series of chemical substances, indicated that some chemicals seemed to be hardly affected while others showed large changes in the levels of biological determinants. Excretion functions seemed to play a key role in the differences observed when simulating occupational exposures for elderly individuals. But parameters as metabolism, storage, long half-lives, hydrophilic or lipophilic property of a chemical, which are

often chemical-specific, also It is therefore essential to study and describe not only some specific chemicals, but also the mechanism underlying those changes in order to be able to forecast changes for new chemicals.

Regarding toxicokinetic differences due to sex, Gochfeld (2007) presented that these changes appear to be about 20 %, mostly in the direction of increased female susceptibility due to body composition. However, he also mentioned that some of the metabolic differences reach an order of twofold. The author stated that there is still a tendency to believe that most sex differences are related to morphology and body size. The main part of this project consisted to quantify the influence of sex on urinary levels of biomarkers of exposure. The human volunteer study also involved an important work in analysis. One of the analytical methods, the determination of urinary 1M2P, had been improved and was calibrated through the new experimental data. In comparison to the former procedure, which included a solid-phase extraction and derivatization before analysis by gas chromatography coupled to a flame ionization detector, the alternative one corresponded to the simple headspace technique, which improved the sensitivity of analysis and lowered the limit of detection. The urinary levels measured for all the studied biological indicators of exposure showed that there exist differences between men and women when exposed to organic solvents which could be explained not only by physiological differences but also by differences in CYP2E1 enzyme activity. The latter one seemed to be “estrogen-sensitive” showing an decrease in activity when estrogen levels are high (Kennedy, 2008). When combining this hypothesis with the results observed, this study suggests that the use of hormonal contraceptive like the combined contraceptive pill increases CYP2E1 activity as the level in free estradiol is lower in women taking the contraceptive pill (Bjørnerem et al., 2004) than in women without any hormonal contraceptive. The only comparable study is the one conducted by

Ernstgård et al. (2003a,b). They studied two organic solvents and had a similar approach, with the difference of exposing human volunteers during two hours of light physical exercise (50 W). Their conclusion was that small differences due to sex had been observed, mainly due to body build. When looking at the measured values after two hours of exposure obtained in this study, the same conclusion could be deduced. Thus exposure time is a determinant factor for underlining toxicokinetic differences due to sex in biological monitoring of exposure.

The whole set of experimental data obtained from the human volunteer study were used in the last part of this project. A general and simple toxicokinetic model (Pierrehumbert et al., 2002), adapted for the three studied organic solvents, allowed to show that metabolic parameters can have an important impact on the urinary levels of the corresponding biomarkers. When simulations were performed with the same values for the metabolic parameters but with the data measured for the female human volunteer groups, results were quite similar to those obtained for men, indicating that physiological parameters have a rather weak influence on the urinary levels of the different biological indicators of exposure. Women with hormonal contraceptive showed values close to the ones determined for men, which was not the case for women without hormonal contraceptive. Thus, metabolic parameters were modified for the latter ones by reducing the Michaelis-Menten maximum rate value by half for each solvent, with the assumption that the differences observed between the human volunteers groups are due to the effect on the CYP2E1 activity by exogenous hormones. Simulations with the modified metabolic parameters values resulted in a better agreement with the experimental data obtained for women without hormonal contraceptive. Moreover the fact that differences were observed between women underlines the idea that toxicokinetic differences mainly involve metabolism, affirmed by Gochfeld (2007).

Another purpose was to explore genetic polymorphism in CYP2E1. The genotype of the human volunteers showed that a minority was carrier of the studied variant alleles, which were CYP2E1*5 and CYP2E1*6. Literature on this subject is very inconsistent. Prieto-Castello et al. (2010) observed for example a reduced activity of CYP2E1 in the presence of CYP2E1*5 and CYP2E1*6 variant alleles. The results in this study had a tendency to show the opposite, which could be explained by the very low number of carriers of the variant allele or by an eventual high interindividual variability in CYP2E1 enzyme activity (Bebia et al., 2004).

In conclusion, this study showed that differences due to age could have an important impact on urinary levels of biological indicators of exposure in function of the chemical considered. But the present simulations did not take into account the apparition of various diseases with age and the increase use of medications, aspects that would probably enhance those observed differences. Disease is in fact a major source of variability in drug response (Löf and Johanson, 1998). The present predictions should therefore be considered as minima to be expected. This study also suggests that differences due to sex could have an important effect on urinary levels of biological determinants. Indeed, experimental data pointed out that for same exposure conditions, men and women can show a difference of 50 % among the urinary biological indicators levels. Actually statistically significant differences were particularly observed between women taking a hormonal contraceptive and those without. Consequently differences should also exist between men and women. Moreover the effect due to the hormonal contraceptive suggests that the use of menopausal hormone therapy in elderly women could have a considerable impact on the toxicokinetics of chemical agents.

Overall these observations give evidence of a major interindividual variability, an aspect that should have its place in the approaches for setting limits of occupational exposure.

5 Perspectives

This study had a special experimental part as human volunteers have participated to it. The experimental protocol followed severe rules and conditions, which made that the whole study focused on a healthy population. Hormonal contraceptive were accepted because it concerns a majority of women today and the recruitment period showed that it was actually difficult to find young women without hormonal contraception. Results showed finally that exogenous hormones can have an effect on the metabolism of organic solvents, which suggest that menopausal hormone therapy, which also concerns a lot of women, could be a subject of concern in the field of occupational health. Moreover, in a general way, this study underlines that the use of medication has to be taken into account in the assessment of occupational exposure. The same is true for physical activity. Indeed, participants were exposed to the studied substances at rest and simulations within working conditions showed that physical activity can have an important effect on urinary levels of biomarkers.

Thus, the results of this project mainly showed that the relation between external and internal exposure to a chemical is not that simple. For same exposure conditions, the interindividual variability can have an important impact on the levels of biological determinants, which leads to think that the approach to fix BEIs on basis of a direct correlation with the TLVs is not appropriate. More information, if possible, about the different BEIs should be given, as for example an annotation when parameters like age, sex or the use of medications can have an important effect on the levels of the biomarkers of exposure.

For the occupational hygienist, the results of the project underline that it is important to couple biological monitoring with air sampling. In fact, the only value of a biological indicator of exposure could lead to an under- or an overestimation of the occupational exposure, which can have a consequence on the workplace risk assessment.

For the occupational physician, the results of the study emphasize that the interpretation of biological monitoring results needs to take into account interindividual variability. For an elder worker, it is important to follow his health status, in particular his renal functions as these could have an influence on the elimination of the chemical compounds. Otherwise, male/female differences as the use of medications, among others hormonal treatments, should be taken into account when evaluating the occupational exposure of a person upon biological monitoring results. In this project, it has been showed that not only physiological parameters (for example body fat) can influence urinary levels of several biological indicators of exposure but also exogenous hormones. The use of hormonal contraceptives can have an effect of about 50 % on the urinary levels of several biomarkers of exposure, a magnitude that can have an important consequence in the interpretation of a biomonitoring result when comparing it to a BEI.

Furthermore, in order to take into account uncertainty around the different parameters used in the TK models, it would be possible to develop them further as population models within a Bayesian framework.

Finally, the main objective of this study was to evaluate the influence of age and sex on the toxicokinetics of solvents. Another concern would be to investigate the influence of such parameters on the toxicodynamics of solvents.

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Annex I – Experimental data

Table 1. Summary of the controlled human exposure sessions performed at the IST

N° expo	Date	Solvant	$N_{volunteers}$	$C_{chamber,average}$ [ppm]
1	04.12.2007	MEK	2	88.6
2	11.12.2007	MEK	2	101.6
3	18.12.2007	1M2P	2	52.5
4	15.01.2008	1M2P	2	48.1
5	29.01.2008	STY	3	10.1
6	05.02.2008	STY	2	10.1
7	12.02.2008	1M2P	2	51.7
8	26.02.2008	111TCE	2	99.2
9	04.03.2008	111TCE	3	99.9
10	11.03.2008	STY	1	10.8
11	18.03.2008	MEK	2	100
12	26.03.2008	111TCE	1	104.9
13	07.07.2008	1M2P	1	55.2
14	22.07.2008	MEK	1	52.3
15	05.08.2008	111TCE	1	102.1
16	30.09.2008	STY	1	9.3
17	13.01.2009	1M2P	3	60.2
18	15.01.2009	1M2P	3	59.2
19	27.01.2009	MEK	3	101.7
20	29.01.2009	MEK	3	103.1
21	03.02.2009	MEK	2	101.6
22	10.02.2009	111TCE	1	111.5
23	12.02.2009	1M2P	1	60.1
24	17.02.2009	MEK	3	100.7
25	19.02.2009	111TCE	2	110.4
26	03.03.2009	1M2P	1	55.2
27	05.03.2009	1M2P	1	52.3
28	10.03.2009	111TCE	2	102.1
29	12.03.2009	1M2P	3	54.5
30	17.03.2009	111TCE	3	101.6
31	24.03.2009	MEK	2	104.9
32	26.03.2009	111TCE	4	103.2
33	06.04.2009	111TCE	1	105.5
34	07.04.2009	1M2P	3	57
35	23.04.2009	MEK	2	105.6
36	29.04.2009	MEK	3	97.4
37	07.05.2009	1M2P	1	52.1
38	13.05.2009	1M2P	1	54.2
39	19.05.2009	1M2P	1	52.3
40	28.05.2009	111TCE	4	105.3
41	16.06.2009	MEK	1	93

Table 2. Characteristics of the human volunteers with their CYP2E1 genotypes

Volunteer	Sex	Age [years]	BW [kg]	BH [cm]	BMI	BF [%]	Cyp*5	Cyp*6	Hormonal contraceptive
1	man	20	75	184	22.15	11.7	c1/c1	TT	
2	man	23	67	176	21.63	10.1	c1/c1	TT	
3	man	25	64	179	19.97	6.2	c1/c1	TA	
4	man	19	69	176	22.28	6.4	c1/c1	TT	
5	man	24	62	173	20.72	9.4	c1/c1	TT	
6	man	22	64	175	20.90	11.5	c1/c1	TT	
7	man	20	74	178	23.36	15.7	c1/c1	TT	
8	man	22	60	175	19.59	9.7	c1/c1	TT	
9	man	25	77	179	24.03	8.7	c1/c1	TT	
10	man	24	95	180	28.70	24	c1/c1	TT	
11	woman	24	68	169	23.81	14.3	c1/c1	TT	YES
12	woman	25	59	173	19.71	18.7	c1/c1	TT	YES
13	woman	23	53	163	19.95	20.2	c1/c1	TT	YES
14	woman	23	51	165	18.73	14.3	c1/c1	TT	YES
15	woman	24	54	167	19.36	22.4	c1/c1	TT	YES
16	woman	19	53	163	19.95	15.2	c1/c1	TT	YES
17	woman	25	64	165	23.51	20.7	c1/c1	TA	YES
18	woman	21	62	172	20.96	19.3	c1/c1	TT	YES
19	woman	21	72	163	27.10	29.1	c1/c1	TT	YES
20	woman	21	65	170	22.49	23.8	c1/c1	TT	YES
21	woman	21	67	163	25.22	28.7	c1/c1	TT	NO
22	woman	26	66	163	24.84	30.2	c1/c2	TA	NO
23	woman	22	58	165	21.30	21.1	c1/c1	TT	NO
24	woman	22	54	160	21.09	14.7	c1/c1	TT	NO
25	woman	31	83	166	30.12	38.2	c1/c1	TA	NO

Table 3. Summary of MEK concentrations in urine for every participant

Volunteer	$C_{MEK,0h}$ [mg/l]	$C_{MEK,2h}$ [mg/l]	$C_{MEK,4h}$ [mg/l]	$C_{MEK,6h}$ [mg/l]	$C_{MEK,8h}$ [mg/l]	$C_{MEK,10h}$ [mg/l]	$C_{MEK,12h}$ [mg/l]	$C_{MEK,14h}$ [mg/l]	$C_{MEK,24h}$ [mg/l]
1	0.08	0.85	1.04	0.96	0.3	0.11	0.04		0.06
2	0.03	1	1.13	0.99	0.23	0.14		0.08	0.06
3	0.11	0.79	1.01	0.93		0.21	0.09		0.04
4	0.05	0.58	1.04	1.04			0.09	0.02	0.06
5	-	0.94	1.22	1.01	0.36		0.09	0.14	0.06
6	0.09	0.82	0.9	0.99	0.39	0.1		0.22	0.15
7	0.09	0.9	1.06	0.88			0.24		0.07
8	0.06	0.9	1.15	1.05		0.3		0.04	0.06
9	0.08	0.76	1.06	1.32		0.55		0.08	0.05
10	0.1	0.66	0.95	0.87	0.52				0.04
11	0.18	1.07	1.35	1.26		0.14		0.1	0.11
12	0.06	0.65	0.72	0.79	0.71				
13	0.1	0.83	0.99	0.96		0.12	0.05		0.05
14	0.05	1.15	1.65	1.49		0.21	0.13	0.1	0.06
15	0.08	0.83	0.96	0.92		0.14		0.07	0.08
16	0.13	0.88	1	0.92		0.18		0.1	0.05
17	0	0.59	0.79	0.75		0.09	0.05		0
18	0.11	0.87	1.01	0.9	0.46				
19	-	0.8	0.79	0.77	0.9			0.09	0.13
20	0	0.63	0.89	0.95	0.43			0.09	0.08
21	-	0.81	1.14	1.09		0.17		0.14	0.09
22	0.06	0.99	1.28	1.35	0.65		0.09	0.05	0.06
23	-	1.13	2.05	2.72	2.73	0.5			0.12
24	-	0.67	1.16	1.33	0.44			0.15	0.07
25	-	0.57	0.72	0.73	0.23			0.15	0.07
Moy_{femme}	0.08	0.83	1.10	1.13	0.82	0.19	0.08	0.10	0.07
SD_{femme}	0.06	0.19	0.37	0.50	0.80	0.13	0.04	0.03	0.03
$Moy_{femme,avec}$	0.08	0.83	1.02	0.97	0.63	0.15	0.08	0.09	0.07
$SD_{femme,avec}$	0.06	0.18	0.28	0.23	0.22	0.04	0.05	0.01	0.04
$Moy_{femme,sans}$	0.06	0.83	1.27	1.44	1.01	0.34	0.09	0.12	0.08
$SD_{femme,sans}$		0.23	0.48	0.76	1.16	0.23		0.05	0.02
Moy_{homme}	0.08	0.82	1.06	1.00	0.36	0.24	0.11	0.10	0.07
SD_{homme}	0.03	0.13	0.09	0.13	0.11	0.17	0.08	0.07	0.03

Table 4. Summary of free 1M2P concentrations in urine for every participant

Volunteer	$C_{1M2P,0h}$ [mg/l]	$C_{1M2P,2h}$ [mg/l]	$C_{1M2P,4h}$ [mg/l]	$C_{1M2P,6h}$ [mg/l]	$C_{1M2P,8h}$ [mg/l]	$C_{1M2P,10h}$ [mg/l]	$C_{1M2P,12h}$ [mg/l]	$C_{1M2P,14h}$ [mg/l]	$C_{1M2P,16h}$ [mg/l]	$C_{1M2P,20h}$ [mg/l]	$C_{1M2P,24h}$ [mg/l]
1	0	1.41	2.55	2.57	1.62	0	0	0	0		0
2	0	1.29	2.26	3.21	2.22		0.31		0		0
3	0	1.42	2.29	3.11	1.18		0.28		0		0
4	0.11	0.85	2.33	2.68	2.32			0.14			0.09
5	0	1.73	2.71	3.69		1		0.29	0	0	0
6	0	1.73	3.07	3.33		1.06	0.37	0.19	0		0
7	0	1.8	2.36	3.37	3.05		0.51				0
8	0	1.52	2.52	2.79	1.56	0.42	0.14		0		0
9	0	0.93	2.08	2.12			0.33				0
10	0	1.85	2.23	-				0			0
11	0	1.02	2.25	2.71	2.05	0.63		0.35	0	0	0
12	0.12	1.11	1.88	2.42	0.94	0.28		0.29			0
13	0	2.91	2.7	2.77		0.34	0.23		0.2		0
14	0.18	1.91	3.84	3.88	1.54	0.39	0.17				0.14
15	0	1.4	1.52	1.22		0.55		0			0
16	0	1.37	1.88	4.16		0.83			0		0
17	0	1.79	2.56	3.05		0.69	0.16		0	0	0
18	0	1.85	2.31	2.67	1.68	0.34			0		0
19	0.19	1.81	2.05	1.44			0.15			0	0
20	0	1.9	2.32	1.78			0.72			0	
21	0	2.09	2.97	2.35		0.27		0			0
22	0	2.39	4.38	4.07	3.5	1.71			0.19		0
23	0.35	1.06	3.48	3.15	6.2		1.24			0.16	
24	0	1.72	3.34	6.6	4.26	0.83				0	
25	-	1.79	2.67	2.86		1.12		0.19			0
Moy_{femme}	0.06	1.74	2.68	3.01	2.88	0.67	0.45	0.17	0.07	0.03	0.01
SD_{femme}	0.11	0.51	0.80	1.32	1.87	0.42	0.45	0.16	0.10	0.07	0.04
$Moy_{femme,avec}$	0.05	1.71	2.33	2.61	1.55	0.51	0.29	0.21	0.04	0.00	0.02
$SD_{femme,avec}$	0.08	0.54	0.63	0.96	0.46	0.20	0.24	0.19	0.09	0.00	0.05
$Moy_{femme,sans}$	0.09	1.81	3.37	3.81	4.65	0.98	1.24	0.10	0.19	0.08	0.00
$SD_{femme,sans}$	0.18	0.50	0.65	1.68	1.39	0.60		0.13		0.11	0.00
Moy_{homme}	0.01	1.45	2.44	2.99	1.99	0.62	0.28	0.12	0.00	0.00	0.01
SD_{homme}	0.03	0.35	0.29	0.49	0.67	0.50	0.16	0.13	0.00		0.03

Table 5. Summary of total 1M2P concentrations in urine for every participant

Volunteer	$C_{1M2P,0h}$ [mg/l]	$C_{1M2P,2h}$ [mg/l]	$C_{1M2P,4h}$ [mg/l]	$C_{1M2P,6h}$ [mg/l]	$C_{1M2P,8h}$ [mg/l]	$C_{1M2P,10h}$ [mg/l]	$C_{1M2P,12h}$ [mg/l]	$C_{1M2P,14h}$ [mg/l]	$C_{1M2P,16h}$ [mg/l]	$C_{1M2P,20h}$ [mg/l]	$C_{1M2P,24h}$ [mg/l]
1	0	1.57	2.74	4.21	2.15	0.23	0	0	0		0
2	0	1.82	2.88	4.1	3.16		1.58		0.77		0
3	0	1.8	3.52	4.46	1.39		0.46		0.22		0.21
4	0.2	1.01	3.03	2.82	2.69			0.25			0.23
5	0	2.36	3.88	8.8		2.21		0.22	0	0	0
6	0	1.85	3.18	3.49		1.52	0.44	0.37	0		0
7	0	2.21	3.15	4.14	3.37	0.81					0
8	0	1.89	3.37	4.2	2.51	0.75	0.34		0.19		0
9	0	1.32	2.57	2.98			0.55				0.17
10	0.12	2.31	2.35	-				0.3			0
11	0.22	1.5	2.07	2.67	2.12	1.01		0.49	0.17	0.25	0
12	0.38	1.71	2.81	3.28	1.46	0.6		0.63			0
13	0.26	3.19	3.07	5.87		0.98	0.23		0.36		0.25
14	0.31	1.77	4.73	6.88	5.39	1.75	0.38				0.49
15	0	3.02	2.78	4.19		1.35		0.23			0
16	0	1.8	1.89	2.85		1.86			0.25		0
17	0	1.85	2.66	6.1		0.93	0.28		0.15	0.1	0
18	0	2.03	2.39	3.83	2.26	0.65			0		0
19	0.27	1.92	3.54	3.65			0.8			0	0
20	0	2.66	3.06	2.37			1.38			0.22	
21	0	2.68	3.31	4.89		0.29		0.32			0.28
22	0	2.95	4.67	4.54	4.25	3.1			0.42		0.31
23	0.41	1.49	4.43	3.69	4.9		1.86			0.31	
24	0	1.61	3.46	6.37	6.86	1.28				0.43	
25	-	2.13	3.13	4.78		2.21		0.64			0
Moy_{femme}	0.13	2.15	3.20	4.40	3.89	1.33	0.82	0.46	0.23	0.22	0.11
SD_{femme}	0.16	0.58	0.87	1.41	2.00	0.79	0.67	0.18	0.15	0.15	0.17
$Moy_{femme,avec}$	0.14	2.15	2.90	4.17	2.81	1.14	0.61	0.45	0.19	0.14	0.08
$SD_{femme,avec}$	0.16	0.59	0.81	1.58	1.76	0.47	0.48	0.20	0.13	0.12	0.17
$Moy_{femme,sans}$	0.10	2.17	3.80	4.85	5.34	1.72	1.86	0.48	0.42	0.37	0.20
$SD_{femme,sans}$	0.21	0.64	0.70	0.97	1.36	1.21		0.23		0.08	0.17
Moy_{homme}	0.03	1.81	3.07	4.36	2.55	1.10	0.56	0.23	0.20	0.00	0.06
SD_{homme}	0.07	0.43	0.46	1.76	0.72	0.77	0.53	0.14	0.30		0.10

Table 6. Summary of TCE concentrations in urine for every participant

Volunteer	$C_{TCE,0h}$ [mg/l]	$C_{TCE,2h}$ [mg/l]	$C_{TCE,4h}$ [mg/l]	$C_{TCE,6h}$ [mg/l]	$C_{TCE,8h}$ [mg/l]	$C_{TCE,10h}$ [mg/l]	$C_{TCE,12h}$ [mg/l]	$C_{TCE,14h}$ [mg/l]	$C_{TCE,16h}$ [mg/l]	$C_{TCE,20h}$ [mg/l]	$C_{TCE,24h}$ [mg/l]
1	-	0.12	1.37	6.43		3.64	0.88	5.59		1.19	2.59
2	0	0.53	2.27	5.91	10.88	9.5	8.89				4.68
3	0	0.35	0.96	6.3			0.24				2.43
4	0.56	0.95	2.52	5.67				3.19			6.63
5	0	2.14	4.31	5.36	5.39		2.18		2.89		3.83
6	0	0.93	2.55	3.55		2.5	5.43		4.75		3.1
7	0	0.62	1.13	6.76			8.26				5.23
8	0.35	1.05	1.13	9.59		5.3		4.68	5.63		6.59
9	0	1.16	4.59	5.97	1.38		4.33				3.82
10	0.54	0.27	0.74	1.05	1.32	6.27					5.67
11	0.19	0.31	1.65	2.33	2.69		2.28	2			4.86
12	-	-	-	-	-	-	-	-	-	-	-
13	0.2	0.48	2.16	3.3	9.79			8.94			7.21
14	0	0.4	1.76	2.77		11.63		5	1.17		1.39
15	0	0.19	-	4.09				4.31			6.8
16	0	0.83	2.27	3.03		10.44	6.34				3.2
17	0.11	0.54	1.02	2.91	5.34		1.78			3.54	8.14
18	0	1.13	2.21	4.73		9.95	2.1	1.48	0.91	3.93	5.33
19	0	0.67	2.73	5.46	7.92		11.08				7.37
20	0	0.64	2.27	5.82	3.99			3.22			3.44
21	0	0.99	0.69	2.25		4.25		1.25			1.47
22	0.05	0.85	1.01	3.41	1.53	3.83		3.51			5.85
23	0	0.13	0.28	0.41	0.88		2.85	0.57			1.59
24	0	-	1.42	3.06		5.84		1.79			2.32
25	-	0.35	0.97	1.32		2.7	1.02				1.7
Moy_{femme}	0.04	0.58	1.57	3.21	4.59	6.95	3.92	3.21	1.04	3.74	4.33
SD_{femme}	0.07	0.31	0.74	1.48	3.31	3.64	3.59	2.46	0.18	0.28	2.47
$Moy_{femme,avec}$	0.06	0.58	2.01	3.83	5.95	10.67	4.72	4.16	1.04	3.74	5.30
$SD_{femme,avec}$	0.09	0.28	0.52	1.26	2.89	0.86	4.02	2.69	0.18	0.28	2.28
$Moy_{femme,sans}$	0.01	0.58	0.87	2.09	1.21	4.16	1.94	1.78			2.59
$SD_{femme,sans}$	0.03	0.41	0.42	1.24	0.46	1.30	1.29	1.26			1.85
Moy_{homme}	0.16	0.81	2.16	5.66	4.74	5.44	4.32	4.49	4.42	1.19	4.46
SD_{homme}	0.25	0.58	1.38	2.20	4.51	2.70	3.43	1.21	1.40		1.55

Table 7. Summary of TCA concentrations in urine for every participant

Volunteer	$C_{TCA,0h}$ [mg/l]	$C_{TCA,2h}$ [mg/l]	$C_{TCA,4h}$ [mg/l]	$C_{TCA,6h}$ [mg/l]	$C_{TCA,8h}$ [mg/l]	$C_{TCA,10h}$ [mg/l]	$C_{TCA,12h}$ [mg/l]	$C_{TCA,14h}$ [mg/l]	$C_{TCA,16h}$ [mg/l]	$C_{TCA,20h}$ [mg/l]	$C_{TCA,24h}$ [mg/l]
1	-	0.03	0.03	0.27		0.22	0.13	0.58		0.21	0.45
2	0	0	0.02	0.08	0.24	0.18	0.3				0.62
3	0	0	0	0.07			0.09				0.13
4	0.49	0.35	0.29	0.79				0.31			0.45
5	0	0.03	0.08	0.28	0.34		0.2		0.47		0.93
6	0	0.03	0.04	0.09		0.09	0.2		0.31		0.47
7	0	0.01	0.02	0.15			0.22				0.68
8	0	0.01	0.02	0.24		0.26		0.41	0.45		0.76
9	0	0	0.02	0.05	0.01		0.15				0.08
10	0	0	0.01	0.02	0.05	0.33					0.75
11	0	0	0.04	0.08	0.1		0.13	0.27			0.51
12	-	-	-	-	-	-	-	-	-	-	-
13	0	0	0.03	0.16	0.31			0.68			0.66
14	0	0	0.02	0.08		0.39		0.28	0.19		0.3
15	0	0	-	0.18				0.49			0.53
16	0	0	0.03	0.08		0.84	1.18				0.62
17	0	0	0.01	0.07	0.14		0.1			0.16	0.38
18	0	0.01	0.06	0.25		0.64	0.19	0.28	0.29	0.69	1.06
19	0	0.01	0.1	0.28	0.53		0.92				1.68
20	0	0.02	0.08	0.24	0.26			0.63			0.88
21	0	0	0	0		0.03		0.03			0.02
22	0	0	0.04	0.18	0.08	0.46		0.35			0.63
23	0	0	0	0.01	0.01		0.06	0.01			0.05
24	0	-	0.06	0.21		0.9		0.58			1.42
25	-	0.02	0.06	0.13		0.54	0.29				0.7
Moy_{femme}											
SD_{femme}											
$Moy_{femme,avec}$	0.00	0.00	0.05	0.16	0.27	0.62	0.50	0.44	0.24	0.43	0.74
$SD_{femme,avec}$	0.00	0.01	0.03	0.08	0.17	0.23	0.51	0.19	0.07	0.37	0.43
$Moy_{femme,sans}$	0.00	0.01	0.03	0.11	0.05	0.48	0.18	0.24			0.56
$SD_{femme,sans}$		0.01	0.03	0.10	0.05	0.36		0.27			0.57
Moy_{homme}	0.05	0.05	0.05	0.20	0.16	0.22	0.18	0.43	0.41	0.21	0.53
SD_{homme}	0.16	0.11	0.09	0.23	0.16	0.09	0.07	0.14	0.09		0.27

Annex II – TK models equations

- **TK model for methyl ethyl ketone**

```
METHOD RK4

STARTTIME = 0
STOPTIME=24
DT = 0.02


{Differential equations}

d/dt (CENTRAL) = + AA - UEc + PtoC - CtoP - UEc - Met
INIT CENTRAL = 0

d/dt (PERIPHERAL) = - PtoC + CtoP
INIT PERIPHERAL = 0


{FLOWS}

{Air absorption rate [mg/h]}
AA = Cexp*Valv*BW**0.7*Rpulm

{Air excretion rate from central compartment [mg/h]}
AEc = CENTRAL*Valv*BW**0.7/(Pblood_air*Vc)

{Urine excretion rate from central compartment [mg/h]}
UEc = CENTRAL*kur*BW**0.82/Vc

{Peripheral to central compartment transfer rate [mg/h]}
PtoC = PERIPHERAL*Qp/Vp

{Central to peripheral compartment transfer rate [mg/h]}
CtoP = CENTRAL*Qp/(Vc)

{Central to metabolism transfer rate [mg/h]}
Met = CENTRAL*Clmet/Vc
```

{FUNCTIONS}

{Total Body Water [kg]}

TBW = If (Sex=0) then (-12.86+0.1757*BH+0.3331*BW)
Else (-2.097+0.1069*BH+0.2466*BW)

{Body Fat [kg]}

BF = bf*BW/100

{Lean Body mass [kg]}

LBM = BW-BF

{Alveolar ventilation [l/(h*kg^0.7)]}
Valv = IF (TIME>6 AND TIME<18) THEN (2.1951*Qc) ELSE Qc

{Apparent volume of the central compartment [l]}
Vc = Pc_blood*FVc*BW

{Apparent volume of the peripheral compartment [l]}
Vp = Pp_c*FVp*BW

{Cardiac output in central compartment [l/(h*kg^0.7)]}
Qc = IF (TIME>6 AND TIME<18) THEN Qc50W ELSE Qcrest

{Cardiac output in peripheral compartment [l/(h*kg^0.7)]}
Qp = BFp*Qc*BW**0.7

{Fraction of cardiac output in peripheral compartment [-]}
BFp = IF (TIME>6 AND TIME<18) THEN BFp50W ELSE BFprest

{Cardiac output in metabolism [l/(h*kg^0.7)]}
Q1 = BF1*Qc*BW**0.7

{Fraction of cardiac output in metabolism [-]}
BF1 = IF (TIME>6 AND TIME<18) THEN BF150W ELSE BF1rest

{Exposition concentration [mg/l]}
Cexp = IF TIME<6 THEN (MW*TLV/Vmol/2/1000) ELSE 0

{Concentration in the central compartment [mg/l]}
Cc = CENTRAL/Vc

```
{Concentration in the peripheral compartment [mg/l]}
Cp = PERIPHERAL/Vp

{Urine concentration in central compartment [mg/l]}
Curc = UEc/(kur*BW**0.82)

{Metabolic clearance for metabolite1 [l/h]}
Clmet = Q1*Clmet_int/(Q1+Clmet_int)

{Intrinsic metabolite clearance for metabolite1 [l/(h*kg)]}

Clmet_int = VM1*BW**0.75/KM1

{Urine excretion rate [l/(h*kg^0.82)]}
kur = 1.848/1000



{INPUTS}

Sex = 0          {0 = man, 1 = woman}
TLV = 200        {Treshold limit value [ppm]}
BW = 70.7        {Bodyweight [kg]; women = 60.1 (with hormonal contraceptive) and 65.6}
                                  {(without hormonal contraceptive); mean value from volunteers}
BH = 177.5       {Bodyheight [cm]; women = 167 (with hormonal contraceptive)and 163.4}
                                  {(without hormonal contraceptive); mean value from volunteers}
bf = 11.3        {Body fat [%]; women = 19.8 (with hormonal contraceptive) and 26.6}
                         {(without hormonal contraceptive); mean value from volunteers}
Qcrest = 18.0    {Cardiac output at rest [l/(h*kg^0.7)]}
Qc50W = 30.8     {Cardiac output at 50 W [l/(h*kg^0.7)]}
Rpulm = 0.558    {Pulmonary retention [-]}


Vmol = 25.45     {Molar volume [l/mol]}

{Volumes of the different compartments expressed as a fraction of BW}

FVc = 1*TBW/BW
FVp = 1*BF/BW


{Different partition coefficients [-]}

Pblood_air = 123
Pc_blood = 0.856
Pp_c= 1.296
```

{Michaelis-Menten maximum rate and constant [mg/(h*kg^0.75)] and [mg/l]}

VM1 = 5.4
KM1 = 1.1

{Fraction of cardiac output in the different compartments at 50W [-]}

BFp50W = 0.06
BF150W = 0.16

{Fraction of cardiac output in the different compartments at rest [-]}

BFpREST = 0.05
BF1REST = 0.26

{Molecular weight of the different substances [g/mol]}
MW = 72

- **TK model for 1-methoxy-2-propanol**

```
METHOD RK4

STARTTIME = 0
STOPTIME=24
DT = 0.02


{Differential equations}

d/dt (CENTRAL) = + AA - AEc - UEc - CtoCONJ - Met
    INIT CENTRAL = 0


d/dt (CONJUGUE) = + CtoCONJ - UE1
    INIT CONJUGUE = 0


{FLOWS}

{Air absorption rate [mg/h]}
AA = Cexp*Valv*BW**0.7*Rpulm

{Air excretion rate from central compartment [mg/h]}
AEc = CENTRAL*Valv*BW**0.7/(Pblood_air*Vc)

{Urine excretion rate from central compartment [mg/h]}
UEc = CENTRAL*kur*BW**0.82/Vc

{Urine excretion rate from conjugated 1M2P compartment [mg/h]}
UE1 = CONJUGUE*kconj

{Central to conjugated 1M2P compartment transfer rate [mg/h]}
CtoCONJ = CENTRAL*CLconj/Vc

{Central to metabolism transfer rate [mg/h]}
Met = CENTRAL*Clmet/Vc


{FUNCTIONS}

{Total Body Water [kg]}
TBW = If (Sex = 0) then (-12.86+0.1757*BH+0.3331*BW)
Else (-2.097+0.1069*BH+0.2466*BW)
```

```
{Body Fat [kg]}
BF = bf*BW/100

{Lean Body mass [kg]}
LBM = BW-BF

{Alveolar ventilation [l/(h*kg^0.7)]}
Valv = IF (TIME>6 AND TIME<18) THEN (2.1951*Qc) ELSE Qc

{Apparent volume of the central compartment [l]}
Vc = Pc_blood*FVc*BW

{Cardiac output in central compartment [l/(h*kg^0.7)]}
Qc = IF (TIME>6 AND TIME<18) THEN Qc50W ELSE Qcrest

{Cardiac output in metabolism [l/h]}
Q1 = BF1*Qc*BW**0.7

{Fraction of cardiac output in metabolism [-]}
BF1 =  IF (TIME>6 AND TIME<18) THEN BF150W ELSE BF1rest

{Exposition concentration [mg/l]}
Cexp = IF TIME<6 THEN (MW*TLV/Vmol/2/1000) ELSE 0

{Concentration in the central compartment [mg/l]}
Cc = CENTRAL/Vc

{Concentration in the conjugated 1M2P compartment [mg/l]}
Cconj = CONJUGUE/Vc

{Urine concentration from central compartment [mg/l]}
Curc = UEc/(kur*BW**0.82)

{Urine concentration from conjugated 1M2P compartment [mg/l]}
Curconj = UE1/(kur*BW**0.82)

{Total 1M2P urine concentration [mg/l]}
Cur_total = Curc + Curconj

{Metabolic clearance for metabolite [l/h]}
Clmet = Q1*Clmet_int/(Q1+Clmet_int)

{Intrinsic metabolite clearance for metabolite [l/h]}
Clmet_int = VM1*BW^0.75/KM1

{Metabolite clearance for conjugated 1M2P [l/(h*kg^0.75)]}
Clconj = ((VM2*BW^0.75/KM2)*Qc)/((VM2*BW^0.75/KM2)+Qc)
```

```
{Partition coefficient [-]}
Pc_blood = Pc_air/Pblood_air

{INPUTS}

Sex = 0          {0 = man, 1 = woman}
TLV = 100        {Treshold limit value [ppm]}
BW = 70.7        {Bodyweight [kg]; women = 60.1 (with hormonal contraceptive) and 65.6}
                                  {(without hormonal contraceptive); mean value from volunteers}
BH = 177.5       {Bodyheight [cm]; women = 167 (with hormonal contraceptive)and 163.4}
                                  {(without hormonal contraceptive); mean value from volunteers}
bf = 11.3        {Body fat [%]; women = 19.8 (with hormonal contraceptive) and 26.6}
                         {(without hormonal contraceptive); mean value from volunteers}
Qcrest = 18.0    {Cardiac output at rest [l/(h*kg^0.7)]}
Qc50W = 30.8     {Cardiac output at 50 W [l/(h*kg^0.7)]}
Rpulm = 0.9      {Pulmonary retention [-]}
Vmol = 25.45     {Molar volume [l/mol]}

{Volumes of the different compartments expressed as a fraction of BW}
FVc = 1*TBW/BW

{Fraction of cardiac output in the different compartments at 50W [-]}
BF150W = 0.16

{Fraction of cardiac output in the different compartments at rest [-]}
BF1REST = 0.26

{Different partition coefficients [-]}
Pblood_air = 12383
Pc_air = 12280

{Urinary excretion rate for conjugated 1M2P [h-1]}
kconj = 0.20

{Urine excretion rate [l/(h*kg^0.82)]}
kur = 1.848/1000

{Michaelis-Menten maximum rate and constant [mg/(h*kg^0.75)] and [mg/l]}

VM1 = 30
KM1 = 30

VM2 = 0.2
KM2 = 95

{Molecular weight of the different substances [g/mol]}
MW = 90.12
```

- **TK model for 1,1,1-trichloroethane**

```
METHOD RK4

STARTTIME = 0
STOPTIME=24
DT = 0.02


{BILANS}

d/dt (CENTRAL) = + AA + PtoC - AEc - CtoP - CtoM1
INIT CENTRAL = 0

d/dt (PERIPHERAL) = - PtoC + CtoP
INIT PERIPHERAL = 0

d/dt (METABOLITE_1) = + CtoM1 - UE1 - Met
INIT METABOLITE_1 = 0


{FLOWS}

{Air absorption rate [mg/h]}
AA = Cexp*Valv*BW**0.7*Rpulm

{Air excretion rate from central compartment [mg/h]}
AEc = CENTRAL*Valv*BW**0.7/(Pblood_air*Vc)

{Urine excretion rate from metabolite1 compartment [mg/h]}
UE1 = METABOLITE_1*ku1*BW**-0.3

{Peripheral to central compartment transfer rate [mg/h]}
PtoC = PERIPHERAL*Qp/Vp

{Central to peripheral compartment transfer rate [mg/h]}
CtoP = CENTRAL*Qp/Vc

{Central to metabolite1 compartment transfer rate [mg/h]}
CtoM1 = CENTRAL*MC1/V1

{Metabolism rate from metabolite1 compartment [mg/h]}
Met = METABOLITE_1*k_M1_M2*BW**-0.3
```

```
{FUNCTIONS}

{Total Body Water [kg]}
TBW = If (Sex = 0) then (-12.86+0.1757*BH+0.3331*BW)
Else (-2.097+0.1069*BH+0.2466*BW)

{Body Fat [kg]}
BF = bf*BW/100

{Lean Body mass [kg]}
LBM = BW-BF

{Molar volume [l/mol]}
Vmol = 25.43

{Alveolar ventilation [l/(h*kg^0.7)]}
Valv = IF (TIME>6 AND TIME<18) THEN (2.1951*Qc) ELSE Qc

{Apparent volume of the central compartment [l]}
Vc = Pc_blood*FVc*BW

{Apparent volume of the peripheral compartment [l]}
Vp = Pp_c*FVp*BW

{Apparent volume of the metabolite1 compartment [l]}
V1 = P_M1_blood*FV1*BW

{Cardiac output in central compartment [l/(h*kg^0.7)]}
Qc = IF (TIME>6 AND TIME<18) THEN Qc50W ELSE Qcrest

{Cardiac output in peripheral compartment [l/h]}
Qp = BFp*Qc*BW**0.7

{Fraction of cardiac output in peripheral compartment [-]}
BFp = IF (TIME>6 AND TIME<18) THEN 0.06 ELSE 0.05

{Cardiac output in metabolite1 compartment [l/h]}
Q1 = BF1*Qc*BW**0.7

{Fraction of cardiac output in metabolite1 compartment [-]}
BF1 = IF (TIME>6 AND TIME<18) THEN 0.16 ELSE 0.26

{Exposition concentration [mg/m3]}
Cexp =  IF TIME<6 THEN (MW*TLV/Vmol/2/1000) ELSE 0

{Concentration in the central compartment [mg/l]}
Cc = CENTRAL/Vc
```

```
{Concentration in the peripheral compartment [mg/l]}
Cp = PERIPHERAL/Vp

{Concentration in expired air from the central compartment [ppm]}
Cac = 1000*Vmol*AEc/(MW*Valv*BW**0.7)

{Creatinine concentration in metabolite1 compartment [mg/g of creatinine]}
Ccr1 = 1000*UE1/(kcr*BW**0.9)

{Urine concentration in metabolite1 compartment [mg/l]}
Ccu1 = UE1/(kur*BW**0.82)

{Partition coefficient central compartment/blood [-]}
Pc_blood = Pc_air/Pblood_air

{Partition coefficient peripheral/central compartment [-]}
Pp_c = Pp_air/Pc_air

{Partition coefficient metabolite1 compartment/blood [-]}
P_M1_blood = P_M1_air/Pblood_air

{Creatinine excretion rate [mg/(h*kg^0.9]}
kcr = 12.06*MWcr/1000

{Urine excretion rate [l/(h*kg0.82^)]}
kur = 1.848/1000

{Intrinsic metabolite clearance for metabolite1 [l/h]}
MCl1 = VM1*BW^0.75/KM1

{Metabolic clearance for metabolite1 [l/h]}
MC1 = Q1*MCl1/(Q1+MCl1)


{INPUTS}

Sex = 0          {0 = man; 1 = woman}
TLV = 200        {Treshold limit value [ppm]}
BW = 70.7        {Bodyweight [kg]; women = 60.1 (with hormonal contraceptive) and 65.6}
                                {(without hormonal contraceptive); mean value from volunteers}
BH = 177.5       {Bodyheight [cm]; women = 167 (with hormonal contraceptive)and 163.4}
                                {(without hormonal contraceptive); mean value from volunteers}
bf = 11.3        {Body fat [%]; women = 19.8 (with hormonal contraceptive) and 26.6}
                        {(without hormonal contraceptive); mean value from volunteers}
Qcrest = 18.0    {Cardiac output at rest [l/(h*kg^0.7)]}
Qc50W = 30.8     {Cardiac output at 50 W [l/(h*kg^0.7)]}
Rpulm = 0.25
```

{Volumes of the different compartments expressed as a fraction of BW}

FVc = 1*TBW/BW
FVp = 1*BF/BW
FV1 = 0.026

{Different partition coefficients [-]}

Pc_air = 2.53
Pblood_air = 2.53
Pp_air = 263
P_M1_air = 8.6

{Urine excretion rate constants in the different compartments [1/(h*kg^-0.3)]}
ku1 = 0.093

{Metabolic rate TCE to TCA [h-1*kg^-0.3]}
k_M1_M2 = 0.069

{Michaelis-Menten maximum rate for metabolite 1 [mg/(h*kg^0.75)]}
VM1 = 0.42

{Michaelis-Menten constants for metabolite 1 [mg/l]}
KM1 = 5.75

{Molecular weight of the different substances [g/mol]}
MW = 133.40
MW1 = 149.40
MWcr = 113.12

Annex III – *Curriculum Vitae*

Catherine Tomicic

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Education

Since 2006	UNIL	**PhD project** at the Institute for Work and Health in Lausanne
2005 - 2007	ETHZ – UNIL	**Master** of Advanced Studies (MAS) in occupational health
1998 - 2004	UNIL - EPFL	**Diploma** in Chemical Engineering

Professional experience

Since 01/2010 **Federal office of public health – Berne, Switzerland**

Scientific collaborator, Division Chemicals, Section Biocides

- Evaluation of product authorization applications
- Human exposure assessment (member of the human exposure expert group – HEEG)

2008 **Archeological service – Berne, Switzerland**

Collaboration with a diploma student

- Subject: cyclododecane exposure in the field of conservation and restoration of art objects. A collaboration was done with Stefanie Bruhin from the laboratory of conservation and restoration

07/2007 **Faculty of Health Sciences at the University of Abomey Calavi - Cotonou, Benin**

Diploma work in the frame of the MAS in occupational health

- Project: Evaluation of a course support in the frame of a training in occupational health

10/2006 **Institute of Environmental Medecine – Unit of Work Environment Toxicology - Karolinska Institutet– Sockholm, Suède**

- Short term mission supported by a COST grant (Action B25)
- Project : learn to use modelling tools by developing a physiologically based pharmacokinetic model for xylene in the researchers group of Professor Gunnar Johanson

Since 2006 **Institute for Work and Health - Lausanne, Switzerland**

PhD student

- Project title: Biological monitoring of chemical exposure - Toxicokinetic differences due to age and sex.
- Approaches applied to study the influence of age and sex on chemical disposition: human volunteer exposures under controlled conditions and toxicokinetic modelling using compartmental models
- Involvement in field activities with the occupational hygienists

Languages

Luxemburgish	mother tongue
French	fluent
German	fluent
English	fluent
Spanish	conversational ability

Publications

Peer-reviewed articles:

Bieler, G., Thorn, D., Huynh, C.K., **Tomicic, C.**, Steiner, U.-C., Yawalkar, N., Danuser, B. (2011) Case report : Acute life-threatening hypersensitivity pneumonitis in a paint controller. Accepted for publication in *Occupational Medicine.* *(*doi: 10.1093/occmed/kqr057)

Tomicic, C., Berode, M., Oppliger, A., Castella, V., Leyvraz, F., Praz-Christinaz, S.M., Danuser, B. Sex differences in urinary levels of several biological indicators of exposure: a human volunteer study. *Toxicology Letters* 202(3), 218-225.

Tomicic, C., Vernez, D., Belem, T., Berode, M. Human mercury exposure associated with small-scale gold mining in Burkina Faso. *International Archives of Occupational and Environmental Health* 84(5), 539-546.

Vernez, D., Wognin, B., **Tomicic, C.**, Plateel, G., Charrière, N., Bruhin, S. (2011) Cyclododecane exposure in the field of conservation and restoration of art objects. *Annals of Occupational Hygiene* 84(4), 371-374.

Tomicic, C., Berode, M. (2010) Sensitive headspace gas chromatography analysis of free and conjugated 1-methoxy-2-propanol in urine. *Analytical and Bioanalytical Chemistry* 396(7), 2709-2714.

Tomicic, C., Droz, P.-O. (2009) Age differences in biological monitoring of chemical exposure: a tentative description using a toxicokinetic model. *International Archives of Occupational and Environmental Health* 82(5), 669-676.

Abstracts:

Tomicic, C., Droz, P.-O., Berode, M., Praz Christinaz, S.M., Castella, V., Danuser, B., 2010. P101-007 Controlled human exposures to organic solvents: Influence of sex on urinary levels of biological indicators. Abstract in *Toxicology Letters* 196S, 39.

Vernez, D., Bruzzi, R., **Tomicic, C.**, Droz, P.-O., Lazor, R., Kupfersmidt, H. (2004) Modelling exposure to waterproofing agents for an epidemiological study of acute respiratory diseases. *International conference X2004: Exposure Assessment in a Changing Environment.*

Report:

Huynh, J., Arial, M., Droz, P.-O., Charrière, N., **Tomicic, C.**, Boillat, M.-A., Riediker, M. (2008) Maladies et causes d'absences dans les services d'entretien des routes = Diseases and reasons for absences in the road maintenance services = Krankheitsfälle und Ursachen der Arbeitsabwesenheiten in den Strassenunterhaltsdiensten. Swiss *federal roads office.*