Université de Montréal

Caractérisation de la variabilité interindividuelle de la toxicocinétique des composés organiques volatils

par

Andy Nong

Département de santé environnementale et santé au travail

Faculté de médecine

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Cette thèse intitulée :

Caractérisation de la variabilité interindividuelle de la toxicocinétique des composés organiques volatils

présenté par :

Andy Nong

a été évaluée par un jury composé de personnes suivantes :

Robert Tardif

Président rapporteur

Kannan Krishnan

Directeur de recherche

Claude Viau

Membre du jury

Dan Krewski

Examinateur externe

Sébastien Sauvé

Représentant du doyen de la FES

RÉSUMÉ

Un facteur de 10 est généralement appliqué en analyse de risque à la santé aux composés organiques volatils (COV) pour prendre en considération les différences toxicocinétiques (TK) et toxicodynamiques (TD) entre les individus d'une population. La valeur de l'élément TK exprimée par le facteur de variabilité interindividuelle (FVI-TK) qui a été suggérée est égale à 3,2 ; cependant, cette valeur qui est utilisée par défaut n'est pas réaliste pour toutes les substances chimiques. L'objectif de cette thèse consiste à développer des outils pour l'estimation du FVI-TK selon la disponibilité des données. Premièrement, pour le cas où seulement les valeurs limites (minimum et maximum) des déterminants pharmacocinétiques sont connues (avec aucune connaissance des distributions statistiques), une approche par bornes de probabilité a été développée. Deuxièmement, pour les situations où les données individuelles des déterminants pharmacocinétiques sont disponibles, elles ont été introduites dans des modèles pharmacocinétiques à base physiologique (PBPK) en reproduisant des simulations de doses internes pour une population. Cette méthode a été testée dans une étude de modélisation avec le toluène et des données individuelles spécifiques sur le cytochrome P-450 2E1, le volume hépatique et le poids corporel chez des enfants.. Troisièmement, lorsque les données pharmacocinétiques d'une substance sont connues chez l'humain, elles peuvent être évaluées par l'approche Markov Chain Monte Carlo ce qui permet de caractériser la distribution pharmacocinétique des déterminants. À partir de ces distributions, l'importance du FVI-TK a été estimée par l'approche conventionnelle de simulation Monte Carlo. Cette analyse a été effectuée avec des données TK portant sur le méthyle éther butyle tertiaire (MTBE). Finalement, l'estimation du FVI-TK a été raffinée en considérant la distribution et la composition d'une population en tenant compte de sous-populations démographiques (ex. femmes enceintes, enfants, personnes âgées). L'estimation du FVI-TK spécifique pour des sous-populations a été réalisée pour la région de Montréal et du Nunavik à partir des données courantes de recensement. Les outils développés dans cette étude facilitent l'estimation d'un FVI-TK en tenant compte de la nature et de la disponibilité des connaissances sur la substance chimique d'intérêt et sur la population ciblée.

Mots clés : modélisation pharmacocinétique de population, modèles PBPK, toxicocinétique, analyse de risque toxicologique, variabilité interindividuelle, facteur d'incertitude, MCMC, Monte Carlo, composés organiques volatiles

ABSTRACT

A factor of 10 is conventionally applied in the health risk assessment of volatile organic chemicals (VOCs) to account for the toxicokinetic (TK) and toxicodynamic (TD) differences among individuals in a population. The TK portion of the interindividual variability factor (IVF-TK) has been suggested to be equal to 3.2 and this default value may not hold true for all chemicals. The objective of this thesis research was to develop scientifically-sound tools for estimating the IVF-TK on the basis of available data. First, for instances where only the minimum and maximum values of pharmacokinetic determinants were available (with no knowledge of statistical distributions), a Probability-bounds approach was developed. Second, in situations where the subject-specific data on pharmacokinetic determinants were available, such data were incorporated within physiologically-based pharmacokinetic (PBPK) models to generate simulations of internal dose at the population level. This approach was demonstrated using toluene as the model substrate and using subject-specific data on cytochrome P-450 2E1, liver volume and body weight of children collected at the Medical College of Wisconsin. Third, when the human pharmacokinetic data for a chemical were available, these data were analyzed using a Markov Chain Monte Carlo approach to characterize the distributions of pharmacokinetic determinants. Using these distributions in a traditional Monte Carlo simulation approach, the magnitude of IVF-TK was estimated. This approach was demonstrated using TK data on methyl *tert*-butyl ether (MTBE) collected in Karolinska Institutet (Stockholm). Finally, the estimation of IVF-TK was refined by considering parameter distributions based on the composition of population, in terms of specific sub-populations (e.g., pregnant women, children, elderly). Such a population-specific estimation of IVF-TK was performed for the Montreal and Nunavik area using the latest census information. The hierarchial tools developed in this project uniquely facilitate the estimation of the IVF-TK depending upon the nature and extent of available data on the chemical as well as the target population.

Keywords : Pharmacokinetics, population modeling, PBPK model, toxicokinetics, health risk assessment, interindividual variability, uncertainty factors, Markov Chain Monte Carlo, Bayesian analysis, volatile organic chemicals

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de tout mon cœur,
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LISTE DES SIGLES ET DES ABRÉVIATIONS

AUC	Aire sous la courbe
C_a	Concentration artérielle
C_{inh}, C_i	Concentration inhalée
C_{max}	Concentration sanguine maximale
C_t	Concentration dans le tissu t
C_v	Concentration veineuse
C_{vt}	Concentration veineuse sortant du tissu t
Cl	Clairance
Cl_h	Clairance hépatique
Cl_{int}	Clairance intrinsèque
COV (VOC)	Composé organique volatil
PC	Coefficient de partage
CYP2E1	Cytochrome 2E1
CYP450, CYP	Cytochrome P-450
dA_{met}/dt (RAM)	Taux de changement de la quantité métabolisée
dA_t/dt	Taux de changement de la quantité dans le tissu t
E	Coefficient d'extraction hépatique
F_{nl}	Fraction équivalente en lipide neutre dans le tissu
F_w	Fraction équivalente en eau dans le tissu
FACS	Facteur d'ajustement chimiquement spécifique
FVI (IVF)	Facteur de variabilité interindividuelle
FVI-TK (IVF-TK)	Facteur de variabilité interindividuelle toxicocinétique
K_p	Constante de perméabilité dermale
Ka	Constante d'absorption orale
Km	Constante d'affinité Michaëlis-Menten
ILSI	International Life Science Institut
ICRP	International Commission on Radiological Protection
IPCS	International Programme on Chemical Safety
IUF	Facteur de variabiltié interindividuelle

IUF-TK	Facteur de variabilité interindividuelle toxicocinétique
LOAEL	Dose minimale avec effet nocif observé (Lowest Observed Adverse Effect Level)
MC	Monte Carlo
MCMC	Markov Chain Monte Carlo
Minmaxmean	Borne de probabilité minimum, maximum et moyenne
MTBE	Méthyle éther butyle tertiaire
NHANES	National Health and Nutrition Examination Survey
NOAEL	Niveau à effet adverse non observable (No Observal Adverse Effect Level)
P_b, $P_{b:a}$, $P_{b:a(app)}$	Coefficient de partage sang:air (apparent)
$P_{o:w}$	Coefficient de partage octanol:eau
P, P_t, $P_{t:b}$	Coefficient de partage tissu:air ou tissu:sang (indice l = foie, s ou m = muscles, f = gras, et r = rapidement perfusés)
$P_{t:a}$	Coefficient de partage tissu:air
$P_{w:a}$	Coefficient de partage eau:air
Bornes-P (P-Bounds)	Bornes de probabilité (Probability Bounds)
PBPK	Pharmacocinétique à base physiologique (Physiological Based Pharmacokinetics)
PD	Pharmacodynamique
PK	Pharmacocinétique
Q_c	Débit cardiaque
Q_l	Perfusion sanguine du foie
Q_p	Ventilation pulmonaire
Q_t	Perfusion sanguine du tissu t
RfC	Concentration de référence
RfD	Dose de référence
SS-BC	Concentration sanguine à l'état stationnaire
SS	État stationnaire
TBA	Butanol tertiaire
TD	Toxicodynamique

TDI	Dose d'exposition journalière tolérable (Tolerable Daily Intake)
TK	Toxicocinétique
TOL	Toluène
USEPA	US Environmental Protection Agency
V_d	Volume de distribution
V_{max}, V_{maxc}	Vélocité maximum du métabolisme
V_t	Volume du tissu t
XYL	m-Xylène

CHAPITRE I

1. INTRODUCTION GÉNÉRALE

1.1. INTRODUCTION

1.1.1. L'analyse de risque en santé publique

L'analyse de risque à la santé, dans le contexte de cette thèse, refère à l'application des connaissances scientifiques disponibles sur une substance toxique en vue d'évaluer la probabilité et l'importance des effets nefastes suite à une exposition humaine (USEPA 1994). Le cadre général d'une analyse de risque comprend l'identification du danger, l'évaluation de l'exposition, l'association de la relation dose-réponse, et la caractérisation du risque. Suite à l'identification des propriétés toxiques de l'agent, les niveaux d'exposition aux contaminants sont déterminés afin de caractériser une dose interne. La dose interne permet d'établir la relation dose-réponse qui associe le niveau d'exposition à la réponse toxique correspondante. Le risque à la santé est évalué selon le niveau d'exposition et la relation dose-réponse. Le risque acceptable à la santé d'une population dépend de la dose interne et du niveau d'exposition aux substances toxiques comme c'est le cas pour les composés organiques volatiles (COV). L'établissement du niveau acceptable de COV dépend de plusieurs facteurs populationnels permetant de protéger la santé publique et surtout celle des individus plus sensibles.

Plusieurs agences environnementales et d'hygiène établissent des seuils acceptables d'exposition comme la dose d'exposition tolérable (TDI) ou la dose (concentration) de référence (RfD). Le seuil d'exposition peut provenir de l'animal ou de l'humain. Ce seuil peut être une dose sans effet nocif observé (NOAEL), une dose minimale entraînant un effet nocif observé (LOAEL) ou encore une dose repère pour un effet significatif (BMD). US Environmental Protection Agency (USEPA 1994) suggèrent d'utiliser une dose limite d'exposition expérimentale ou extrapolée à partir du système biologique le plus sensible (par exemple : rat, souris, lapin) afin d'établir une RfC pour les COV. Des facteurs sont introduits dans le calcul de la RfC pour considérer l'incertitude et la variabilité (**voir figure 1-1**). Les éléments d'incertitude et de variabilité peuvent correspondre à l'extrapolation de la dose de l'animal à l'humain (variabilité inter-espèce), aux différences biologiques au sein d'une population (variabilité interindividuelle), à

l'extrapolation de haute à faible dose d'exposition, à l'utilisation de valeurs sub-chroniques, et à l'ajustement pour la qualité de l'étude. Le **tableau 1-1** présente une série de facteurs d'incertitudes et de variabilité utilisés par différents organismes environnementaux. Lorsque l'information pertinente sur l'incertitude ou la variabilité de la valeur limite n'est pas disponible, un facteur conservateur de 10 est appliqué. Par exemple, un NOAEL déterminé par des études de toxicité chez le rat est divisé par un facteur de 100 (10X10) pour combler l'absence d'information appropriée sur la variabilité inter-espèce et la variabilité interindividuelle. Dans le doute, le facteur de 10 utilisé par défaut ce qui par prudence contribue à abaisser la valeur limite d'exposition. La suite de ce chapitre présente un aperçu de la caractérisation de la variabilité d'une population par divers facteurs dans le contexte de l'analyse des risques environnementaux à la santé publique liés aux COV.

1.1.2. L'évolution des facteurs d'incertitude et de variabilité

Renwick (1993) a proposé de subdiviser les facteurs de variabilité inter-espèce et interindividuelle en des composantes toxicodynamiques et toxicocinétiques (voir **figure 1-2**). La variabilité d'une population (inter-espèce ou interindividuelle) peut être expliquée par les différences de distribution de la substance dans le corps (toxicocinétique TK) et par les variations au niveau de ses effets toxiques (toxicodynamique TD). Le facteur de variabilité interindividuelle serait divisé également en composantes TK et TD (10 = 3,2 x 3,2), alors que le facteur de variabilité inter-espèce serait divisé par 4,0 ($10^{0.6}$) pour la TK et 2,5 ($10^{0.4}$) pour la TD. Adopté par plusieurs organismes environnementaux appartenant à l'International Programme on Chemical Safety (Meek *et coll.* 2002), la subdivision des facteurs TK et TD permet aussi de dériver un facteur de variabilité exprimé à partir de connaissances populationnelles sur la cinétique ou la toxicité de substances tels que les COV.

Les substances peuvent être définies par les connaissances spécifiques de variabilité qui s'y rapportent afin de dériver un facteur d'ajustement chimiquement spécifique (FACS) tel que proposé par Dorne et Renwick (2005). L'approche quantitative sert à estimer un

facteur de variabilité (inter-espèce ou interindividuelle) d'après l'évidence scientifique populationnelle TK ou TD d'une substance. Ce nouveau facteur représentant la distribution actuelle de la TK au sein d'une population pour une substance précise premet de remplacer la valeur défaut. Renwick a étudié exhaustivement les FACS pour plusieurs substances médicamenteuses et il a déterminé que les FACS pour certains médicaments diffèrent du facteur de 3,2 utilisé par défaut (Renwick et Lazarus 1998). Le FACS assure un niveau de protection spécifique pour une substance et permet de caractériser la variabilité d'une population. En fonction de ces critères, IPCS a développé un guide général sur la détermination de FACS pour les effets toxiques de toutes substances (2005).

1.1.3. La perception sur la variabilité interindividuelle de population

La disponibilité des connaissances sur la toxicité des COV varie d'une substance à l'autre. Représentant une série de substances qui s'évaporent facilement, les COV comprennent des substances aromatiques (benzène, toluène, xylène) et des hydrocarbures halogénés (tetrachloroéthylène, chloroforme). La toxicité de ces substances est neurologique et cancérogène. Les COV se retrouvent dans diverses sources comme des matériaux de constructions, colles, nettoyants, peintures, produits domestiques, décolorants et photocopieurs (Wallace 1991, Wallace *et coll.* 1991). L'utilisation répandue des COV et leur diffusion dans l'environnement représentent un risque pour la population en générale. Comme une population comprend des individus qui présentent une extrême variabilité physiologique et anatomique, la dose interne d'une substance toxique peut différer d'une personne à l'autre. La variation de la dose peut résulter en divers effets toxiques selon les personnes exposées. Dans la perspective d'une analyse de risque des COV pour une population générale, un survol des différentes approches pour caractériser la variabilité de la dose interne d'une population sera présenté en fonction des connaissances disponibles dans la littérature.

De nombreuses approches populationnelles ont été développées à partir des connaissances des processus cinétiques des médicaments chez l'humain. Ces approches

sont applicables aux substances toxiques. Récemment, Dorne *et coll.* (2005) ont évalué la variabilité pharmacocinétique de plusieurs médicaments selon les différents processus impliqués (voies d'absorption, d'élimination, etc ...). Les auteurs ont développé des facteurs de variabilité calculés à partir des différences biologiques dans les processus pharmacocinétiques d'une population. Les médicaments sont ainsi regroupés par voies cinétiques semblables d'où un facteur de variabilité est dérivé.

Plusieurs auteurs ont étudié les caractéristiques physiologiques et anatomiques de diverses populations humaines. L'International Commission on Radiological Protection (ICRP 1975, 2002) a répertorié dans la littérature des séries de valeurs de références physiologiques, anatomiques, et métaboliques chez l'humain de différents groupes d'âges dans la population américaine et européenne. Cette énorme recherche a pour but de déterminer des valeurs biologiques de référence afin d'analyser le risque associé aux substances radioactives. Par contre, la distribution des données est souvent limitée à la moyenne sans mention de l'écart type ou du type de distribution. D'autres ouvrages de référence comme ceux de Hattis (1987), Arms et Travis (1988), Davies et Morris (1993), ILSI (1994) et Brown *et coll.* (1997) sont généralement restreints à des valeurs moyennes pour une population adulte. L'utilisation de ces données est limitée aux cas d'exposition reliés exclusivement aux adultes, comme dans un environnement de travail.

D'autres études ont été produites pour des distributions de paramètres physiologiques pour la population générale tout en considérant en plus d'autres sous-groupes particuliers de la population tels que les enfants, les personnes âgées, ou les femmes enceintes. USEPA (1997a) a réalisé une étude détaillée sur la distribution statistique des facteurs biologiques impliqués dans l'évaluation de l'exposition pour la population nord américaine. Plusieurs sous-groupes de population sont représentés tels que les enfants, les hommes et femmes de tous âges, et les femmes enceintes. Le document sur les facteurs d'exposition comprend une revue de la littérature sur une variété d'études qui ont examiné la distribution des mesures physiologiques en relation avec l'exposition aux contaminants (par exemple : le poids corporel, le taux d'inhalation ou le taux de consommation d'eau). Ces informations sont utilisées dans les approches probabilistes

qui estiment statistiquement des facteurs de variabilité pour le calcul de la dose de référence (Swartout *et coll.* 1998, Finley *et coll.* 2003, Maddalena *et coll.* 2004). Cependant, ces méthodes négligent les aspects cinétique et dynamique des substances. Ainsi, les déterminants biologiques concernant les mécanismes d'action ou la cinétique des substances telles que les différences métaboliques ou physiologiques (débits ou volumes tissulaires, compositions sanguines) ne sont pas abordés dans le document de référence sur l'exposition par l'USEPA.

Afin de considérer l'aspect mécanistique de la cinétique des substances toxiques, Price *et coll.* (2003a) ont récemment étudié la distribution de mesures physiologiques à partir d'un sondage sur la santé de 30 000 habitants au États-Unis (National Health and Nutrition Examination Survey III, NHANES III). Avec cette étude, les auteurs ont développé une base de données (P^3M, Linea, Inc) permettant de repérer la distribution de déterminants pharmacocinétiques comme le débit cardiaque, le volume hépatique ou le poids corporel. La base de données est limitée à la population des États-Unis et exclue les nouveau-nés de moins de 2 mois, les femmes enceintes et les personnes malades. L'étude sur les déterminants physiologiques ne comprend pas les différences métaboliques ou celles au niveau de la composition sanguine dans la population. Lipscomb *et coll.* (2000, 2002, 2003) ont examiné la variabilité des voies l'élimination des substances toxiques chez l'adulte. Ils ont regardé l'écart affectant les enzymes métaboliques et la fonction rénale entre plusieurs adultes de différents âges. Les travaux de référence présentés décrivent la distribution physiologique dans la population en indiquant la relation possible avec la variabilité interindividuelle de la TK.

Cependant, la variation des facteurs mécanistiques (ex. physiologiques, métaboliques) doit être considérée dans son ensemble pour mieux évaluer l'amplitude de la variabilité de la dose interne dans une population.

1.1.4. Les connaissances sur la variabilité interindividuelle toxicocinétique

De nombreuses études et revues ont étudié l'influence de la variabilité des déterminants de la TK des produits chimiques pour divers sous-groupes de la population comme les enfants, adultes, personnes âgées, les femmes enceintes ou allaitantes. Entre autres, Löf et Jonhanson (1998) et Clewell *et coll.* (2002) ont examiné l'effet de la variabilité affectant les déterminants de la TK entre les différents individus d'une population. Ils ont remarqué la dépendance avec l'âge de caractéristiques spécifiques aux processus de la TK pour quelques substances. Un résumé des caractéristiques physiologiques des différents sous-groupes d'une population est présenté au **tableau 1-2**. Ces variations anthropologiques résultent des différences au niveau du développement des individus dans une population. Par exemple, l'immaturité de certains organes chez les enfants ou les altérations affectant certains systèmes biologiques chez les personnes âgées peuvent se traduire par des doses internes différentes de celles rencontrées normalement.

D'autres études sur la distribution des déterminants pharmacocinétiques se sont concentrées sur des sous-groupes spécifiques de la population. La physiologie des femmes enceintes a été examinée pour mieux connaître le développement du fœtus. Des données sur la femme enceinte et le développement prénatal de l'enfant prénatale sont disponibles (Young *et coll.* 1997, O'Flaherty 1998, Faustman *et coll.* 2000, ICRP 2002, Corley *et coll.* 2003), mais l'information est limitée à des valeurs moyennes. La progression du stade prénatale cause plusieurs changements physiologiques et hormonaux jusqu'à la naissance de l'enfant. Notamment, le développement du placenta et la redistribution des débits sanguins et des volumes tissulaires entraînent des modifications pouvant affecter les processus de la TK. Des modèles mathématiques décrivant la cinétique du warfarine ou du méthylmercure chez la femme enceinte ont été développés (Luecke *et coll.* 1997, Corley *et coll.* 2003). L'exposition à des substances toxiques chez la femme allaitante a aussi été explorée (Shelly *et coll.* 1988, Byczkowski 1994, Fisher *et coll.* 1997). Ces études se sont généralement consacrées aux risques d'exposition des nouveau-nés ou des foetus, mais la TK des adultes et celle des femmes enceintes n'a pas encore été comparée.

Plusieurs travaux traitent des différences physiologiques entre les adultes et les enfants; entre autre, l'influence de la croissance des enfants sur l'exposition à des substances toxiques y a été étudiée (Rodman *et coll.* 1993, USEPA 2002, Braum *et coll.* 2003). Le développement physiologique et métabolique du nouveau-né jusqu'à l'adolescent est bien documentée (Haddad *et coll.* 2001, Hines et McCarver 2002, McCarver et Hines 2002, Jonhsrud *et coll.* 2003, Alcorn et McNamara 2003a, 2003b, 2003c). Daston *et coll.* (2004) et Ginsberg *et coll.* (2004b) ont examiné les différences pharmacocinétiques pour des substances médicamenteuses entre les enfants et les adultes. Ils ont observé que l'immaturité biologique de l'enfant engendre une différence au niveau de la pharmacocinétique des médicaments. En principe, l'effet du développement chez l'enfant sur la pharmacocinétique des médicaments devrait être applicable aux substances toxiques comme les COV. Avec les connaissances sur le développement de l'enfant, la modélisation TK permet d'extrapoler les données TK en fonction de l'âge (Pelekis *et coll.* 2001, Price *et coll.* 2003a, de Zwart *et coll.* 2004, Ginsberg *et coll.* 2004a).

Par ailleurs, des études chez les personnes âgées ont aussi été produites. Les différences dans la pharmacocinétique des substances médicamenteuses entraînées par la vieillesse ont été révisées par les travaux de Cusack (2004) et Shah (2004). Ces études décrivent le déclin de certaines fonctions physiologiques du corps avec l'âge. En plus de la réduction du débit cardiaque, la diminution de la clairance rénale et métabolique se démarque dans ce groupe d'âge. Cependant, très peu de travaux ont documenté l'effet du vieillissement sur la TK de substances toxiques comme les COV.

En contrepartie, les données sur la distribution des valeurs des déterminants pharmacocinétiques pour divers sous-groupes de population peuvent servir à la modélisation de la variabilité dans une population. Des modèles mathématiques décrivant les processus physiologiques sont utilisés pour prédire la TK d'une substance chez un individu. Comme les données utilisées dans l'analyse du risque des substances toxiques proviennent souvent de l'animal, ces modèles permettent d'extrapoler à l'humain en supposant que la TK de la substance soit comparable entre ces espèces.

Deux catégories de modèle pharmacocinétique permettent de simuler le devenir d'une substance: les modèles pharmacocinétiques compartimentaux (PK) et les modèles pharmacocinétiques à base physiologique (PBPK). De nombreux modèles PBPK ont été développés pour décrire la TK de diverses substances toxiques comme les COV. Une description détaillée des modèles PBPK est présentée au chapitre suivant. Les modèles PBPK sont normalement utilisés pour décrire la TK chez un individu type ; par ailleurs plusieurs approches permettent de simuler et d'estimer la variabilité pharmacocinétique chez une population.

1.1.5. Les approches populationnelles de modélisation PBPK

La méthode conventionnelle de modélisation pharmacocinétique chez une population est basée sur l'utilisation de valeurs moyennes pour les déterminants du modèle. Le modèle de population moyenne ignore la variabilité au sein de la population et suppose que la cinétique d'une substance est comparable chez tous les individus. Cette méthode générale manque d'efficacité pour prédire la TK chez différents individus incluant les plus susceptibles (Spear et Bois 1994, Krishnan et Johanson 2005). Par conséquent, des approches probabilistes ont été développées pour modéliser la pharmacocinétique d'une population. Il est sous-entendu que les paramètres définissant le modèle sont représentés sous forme de distributions de probabilité. Il existe des méthodes probabilistes qui permettent d'estimer la variabilité des déterminants pharmacocinétiques selon l'étendue des doses internes mesurées dans une population. Ces méthodes d'estimations de la variabilité sont souvent appliquées dans la détermination de la variabilité au niveau de la pharmacocinétique des médicaments. Diverses autres approches probabilistes simulent la distribution de doses internes à partir des connaissances sur la distribution des paramètres physiologiques d'une population. Ces approches de prédiction utilisent des modèles PBPK. Un résumé des études sur la modélisation PBPK de la variabilité interindividuelle de population est présenté dans le **tableau 1-3**. La simulation Monte Carlo est l'approche probabiliste qui a été la plus utilisée en modélisation de la variabilité TK d'une population.

La simulation Monte Carlo (MC) provient des méthodes mathématiques d'échantillonnage aléatoire des valeurs. L'approche consiste en calculs répétitifs où les valeurs des paramètres sont désignées par leur distribution statistique. La modélisation PBPK populationnelle applique la simulation MC : une série de simulations itératives provenant d'un modèle PBPK d'un individu est réalisée et pour chacune de ces simulations, les déterminants physiologiques sont définis par leur distribution statistique dans la population. Après de nombreuses simulations individuelles, la distribution des concentrations internes générées par le modèle PBPK permet de caractériser la variabilité associée à population. L'USEPA (1996) a étudié l'approche MC pour décrire la variabilité TK dans une population. Suite à une consultation avec des experts dans le domaine, l'agence a adopté des lignes directrices sur l'application des simulations MC et la présentation des résultats (USEPA 1997). Plusieurs recommandations ont été suggérées : présenter une analyse de sensibilité des paramètres du modèle afin de caractériser la variabilité TK dans la population avec les déterminants marquants ; donner le détail sur les données de la population pour les distributions des paramètres ; faire une présentation claire et informative des figures de distribution de probabilité des déterminants de la TK ou des doses internes calculées en incluant les données statistiques ; discuter des corrélations possibles entre les paramètres du modèle ; et analyser la stabilité de la simulation MC afin d'assurer la puissance statistique des résultats en fonction du nombre d'itérations et de l'intervalle de confiance (Burmaster et Anderson 1994). Ces suggestions mettent l''emphase sur les données utilisées pour les distributions statistiques du modèle et l'approche MC, ainsi que sur la présentation de ces distributions. Dans la plupart des études présentées au **tableau 1-3** Les distributions de déterminant du modèle proviennent des données échantillonnées dans un sous-groupe de population ou de distributions hypothétiques basées sur l'expertise scientifique des chercheurs. Une fois que ces distributions sont établies, des facteurs de variabilité interindividuelle peuvent être estimés par la différence de la dose interne simulée avec l'approche MC entre l'individu moyen et le plus susceptible. Dans la littérature, on retrouve plusieurs exemples d'application de la modélisation PBPK avec la simulation MC pour l'évaluer la variabilité TK dans une population après une exposition à différentes substances : Droz *et coll.* (1989a, 1989b), Portier et Kaplan (1989), Gearhart

et coll. (1993), Thomas *et coll.* (1996), Clewell *et coll.* (1999), Sweeney *et coll.* (2001). À l'exception de quelques études qui ont recherché l'impact d'une variabilité entre les adultes et les enfants, la plupart des simulations MC réalisées ont été généralement limitées à une population adulte.

De récents développements en modélisation probabiliste visent à promouvoir l'utilisation des approches bayésiennes. La théorie de Bayes suppose que les observations expérimentales peuvent être décrites par une relation statistique entre des données pharmacocinétiques antérieures (*a priori*) et de nouveaux paramètres correspondants (*a posteriori*) (Wakefield 1996, Gelman et Rubin 1996). Ainsi, les distributions *a posteriori* tiennent compte non seulement des données existantes mais aussi des connaissances *a priori*. La modélisation PBPK bayésienne consiste à optimiser les déterminants physiologiques du modèle en fonction de la distribution des paramètres *a priori* et des données expérimentales telles que les concentrations sanguines en fonction du temps. En plus des valeurs statistiques de population, l'approche bayésienne permet d'obtenir des paramètres individuels. La simulation Markov Chain Monte Carlo (MCMC) est la technique la plus souvent utilisée en modélisation PBPK bayésienne (Gelman et Rubin 1996). Les distributions de déterminants *a posteriori* peuvent être utilisées dans la simulation MC du modèle PBPK afin de caractériser la variabilité d'une population. Par exemple, Jonsson *et coll.* (2001) ont déterminé avec la simulation MCMC des valeurs de distribution *a posteriori* pour des paramètres physiologiques et biochimiques à partir de données *a priori* et de données expérimentales provenant d'individus exposés au dichlorométhane pendant un léger exercice, comparable à ce que l'on retrouve dans un milieu de travail. À partir des distributions de déterminants *a posteriori*, des simulations MC peuvent générées afin d'établir la variabilité interindividuelle extrapolée pour une population. Malgré la puissance statistique de la simulation MCMC, cette approche en développement reste à être validée plus efficacement. La simulation MCMC demeure néanmoins une technique efficace qui combine les connaissances actuelles et des données existantes pour définir de nouveaux paramètres utilisés dans la détermination de la variabilité TK dans une population.

Les simulations probabilistes sont pratiques pour déterminer la variabilité de la TK d'une population, mais elles présentent des limites. La nature aléatoire de la simulation MC peut mener à des combinaisons irréalistes des déterminants ce qui génère des résultats improbables. L'utilisation des distributions de déterminant dans les simulations requière aussi une certaine quantité d'informations statistiques telles que la moyenne, l'écart type, la valeur maximale et minimale, et la forme de la distribution (Ferson 1996). Lorsqu'il a un manque d'information sur les distributions de déterminants, des hypothèses sont nécessaires lesquelles peuvent affecter la précision du modèle. La corrélation des paramètres du modèle entre eux peut avoir une influence sur la simulation probabiliste. Par exemple, si $A \times B = C$, on ne peut pas résoudre B en utilisant la formule $B = C / A$ avec une simulation MC à cause de la dépendance des paramètres B et C. La dépendance entre les paramètres et le résultat simulé exige la déconvolution du calcul probabiliste mais celle-ci n'est pas considérée dans la simulation MC (Burmaster *et coll.* 1995). Ces limites dépendent surtout des données qui définissent les déterminants du modèle probabiliste. La manière de traiter les connaissances de populations aura une conséquence sur la robustesse de la caractérisation de la variabilité interindividuelle et ainsi sur l'analyse de risque à la santé publique.

1.2. PROBLÉMATIQUE

Les approches probabilistes présentées permettent de caractériser la variabilité interindividuelle de la TK en intégrant la distribution statistique populationnelle des déterminants pharmacocinétiques dans un modèle PBPK. Par conséquent, les simulations probabilistes dépendent des données populationnelle introduites. Malheureusement, on peut remarquer que la plupart des données pharmacocinétiques de variabilité de population concernent les substances médicamenteuses. De plus, la majorité des travaux existants sur les modèles de substances toxiques visent uniquement les adultes et non sur la population générale composée entre autres d'enfants, de personnes âgées et de femmes enceintes. Comme on peut le constater dans les sections précédentes, l'information populationnelle varie non seulement de l'étude d'une substance à l'étude d'une autre

mais aussi entre les différents sous-groupes d'une population (par exemple, enfants et adultes). Afin d'établir la variabilité interindividuelle de la TK des COV, une gestion appropriée des renseignements connus sur une substance et sur la population ciblée est nécessaire.

Dans une analyse de risque à la santé publique plus critique, on caractérisera l'importance de la variabilité interindividuelle au sein d'une population générale (enfants, adultes, personnes âgées, femmes enceintes) à l'aide des connaissances acquises. Ainsi, cette thèse de recherche traite de l'utilisation de la modélisation PBPK de population pour les COV selon la disponibilité des données populationnelles sur les déterminants physiologiques, biochimiques et physico-chimiques ce qui n'a pas été fait pour les COV.

1.3. OBJECTIFS

L'objectif de cette thèse est de développer des approches pour caractériser l'impact de la variabilité interindividuelle sur le devenir des COV chez une population dans le contexte de l'analyse de risque en santé publique.

En fonction des données de population recueillies, nous définirons l'approche probabiliste la plus appropriée pour caractériser la variabilité interindividuelle par un facteur représentatif de la population simulée. Dans les chapitres suivants, on évaluera 4 types de données connues pour développer l'approche adaptée à la détermination d'un facteur de variabilité interindividuelle pour une population exposée aux COV. Les objectifs spécifiques sont d'évaluer :

1. Une série de données pauvres ou manquantes en information populationnelle;
2. Une série de données individuelles riches en information;
3. Une combinaisons de connaissances venant de diverses études actuelles et publiées;
4. Des statistiques démographiques d'une population véritable en plus de données de populations spécifiques.

Les nouvelles approches développées dans cette thèse ont pour but de faciliter la détermination de la variabilité interindividuelle non seulement selon la nature des données mais aussi par la prise en compte des connaissances sur la substance et sur la population à l'étude.

1.3.1. Organisation de la thèse

L'approche de modélisation PBPK et son utilisation pour l'analyse de risque des COV est décrite dans le chapitre 2. Ce chapitre décrit en détail les notions de base de la pharmacocinétique, les déterminants physiologiques, physico-chimiques et biochimiques du modèle PBPK, et les applications du modèle pour l'analyse du du risque toxicologique des COV. Cette introduction au modèle PBPK présente les connaissances de base de la modélisation et une compréhension de son utilité dans la prédiction de la dose interne pour l'analyse de risque.

Tout d'abord, dans la première étude qui est présentée au chapitre 3, une approche de calcul par bornes de probabilité (Probability-bounds) a été développée pour le cas où seulement certaines informations sur des déterminants TK sont connues (comme la valeur minimum et maximum). Cette approche consiste à faire des calculs de doses internes en se basant sur les limites de confiance inférieures et supérieures d'une distribution de probabilité des déterminants de la TK (Ferson 1989), appelées limites de probabilité (bornes-P). À l'aide d'un algorithme de la concentration sanguine à l'état stationnaire (Andersen 1981, Pelekis et coll. 1997), on a estimé des facteurs de variabilité interindividuelle à partir des bornes-P de la dose interne d'une population adulte exposée au benzène, au tetrachlorure de carbone, au chloroforme, ou au méthylchloroforme. Des exemples de calculs des bornes-P sont présentés en modifiant les propriétés statistiques sur la distribution des déterminants ainsi que l'impact de ces changements sur la caractérisation de la variabilité TK interindividuelle.

La deuxième étude représente une situation où les données individuelles sur la TK des déterminants sont disponibles, tel que présenté au chapitre 4. Cette étude introduit des données riches en valeurs individuelles dans des modèles PBPK pour générer la distribution de doses internes d'une population. Il s'agit d'une approche de modélisation PBPK individuelle pour le toluène qui utilise des données sur la concentration de cytochrome P450 2E1 hépatique (CYP2E1, une importante enzyme métabolisant le toluène) mesurées chez un groupe de 116 enfants de divers âges. Ensuite, la variabilité interindividuelle des enfants étudiés a été caractérisée. Ce projet a été possible grâce à la collaboration du groupe de recherche du Dr Ronald Hines du Medical College de Wisconsin qui a recueilli les données individuelles de concentration en CYP2E1 hépatique, de volume hépatique et de poids corporel chez les enfants.

La troisième étude qui figure au chapitre 5 présente une modélisation avec des données provenant de diverses sources connues sur la TK chez l'humain pour caractériser les distributions de déterminant par l'approche MCMC. Ensuite, l'importance du facteur de variabilité interindividuelle a été estimée à partir de la simulation MC avec les distributions de déterminants *a posteriori*. On a utilisé la simulation MCMC avec des données TK relatives au méthyle éther butyle tertiaire (MTBE) recueillies à Karolinska Institutet en Suède. Ce projet est le fruit d'une collaboration avec le groupe de recherche du Dr. Gunnar Johanson à Karolinska Institutet qui a fourni les données expérimentales et les données dites *a priori*. Le Dr Fredrik Jonsson de l'université d'Uppsala en Suède nous a présenté les techniques et outils de simulation MCMC. Avec les apprentissages résultant de ce stage de recherche, on a combiné les connaissances de plusieurs études sur la TK du MTBE avec l'approche MCMC pour déterminer la distribution des déterminants correspondants aux données actuelles, des données servant à caractériser la variabilité d'une population. Ce projet de stage à Karolinska Institutet sous le parrainage du Dr Johanson a été rendu possible grâce à la bourse de voyage du Département de santé environnementale et santé au travail.

Dans la quatrième étude présentée au chapitre 6, l'estimation d'un facteur de variabilité interindividuelle relative à la distribution et à la composition démographique d'une

population a été étudiée en fonction des caractéristiques spécifiques des déterminants de différents sous-groupes d'âge (par exemple les femmes enceintes, les enfants, adolescents, adultes et personnes âgées). Pour cette approche, nous avons réalisé des simulations MC à partir d'un modèle PBPK pour le toluène. Ces simulations sont spécifiques à chaque sous-groupe de la population dont les données de recensement sur la répartition démographique des populations de la région métropolitaine de Montréal et de la région du Nunavik proviennent de Statistiques Canada. L'objectif de cette étude est de déterminer la dose interne chez les sous-groupes d'individus d'une population générale correspondant à une personne typique et à l'individu plus susceptible. Par ailleurs, on a exploré la sensibilité de la taille des différents sous-groupes de personnes sur la distribution de dose interne de population générale. Ce dernier travail raffine l'analyse probabiliste MC des modèles PBPK populationnelle en considérant la variabilité interindividuelle spécifique des différents individus dans une population hétérogènes.

Les approches présentées dans cette thèse permettent de caractériser les différences toxicocinétiques dans une population à partir des données scientifiques. La modélisation PBPK est uniquement exploitée pour prédire la variabilité interindividuelle en introduisant les différences physiologiques d'une population. Selon les données disponibles, différentes méthodes de modélisation sont développées afin de caractériser la différence de la dose interne au sein d'une population. Les outils développés contribuent à l'enrichissement d'une analyse de risque aux contaminants environnementaux tels que les composés organiques volatils pour établir des niveaux d'exposition sécuritaire à la santé publique.

Tableau 1-1 Principaux facteurs d'incertitudes et de variabilité utilisés par différents organismes environnementaux.

Facteur d'incertitude et de variabilité	Organismes		
	Santé Canada	USEPA	IPCS
Interindividuelle	1 - 10	10	10 (3,2 x 3,2)
Inter-espèce	1 - 10	10	10 (4,0 x 2,5)
Subchronique à chronique		≤10	
Haute à faible dose (LOAEL à NOAEL)		≤10	
Donnée manquante	1 - 100	≤10	1 - 100
Facteur de modification	1 - 10	1 - 10	1 - 10

Dourson *et coll.* (1996).

Tableau 1-2 Résumé des caractéristiques physiologiques de différents sous-groupes de population.

	Absorption	Distribution	Métabolisme	Élimination
Enfants	rythme respiratoire et cardiaque élevés, développement variable de la flore gastro-intestinale	faible volume lipidique	expression immature de certains enzymes, développement de nouvelles voies métaboliques	développement de la voie rénale et hépatique
Adulte			polymorphismes, phénotypes	
Personnes âgées	diminution du débit cardiaque	diminution de la masse musculaire	diminution de l'activité enzymatique	diminition des capacités rénale et hépatique
Femmes enceintes	répartition du débit cardiaque	augmentation des volumes lipidique et placentaire, rétention d'eau		

Tableau 1-3 Exemples d'études de modélisation PBPK sur la variabilité interindividuelle de population.

Approche probabiliste	Population évaluée	Substance	Références
Individuelle en fonction de l'âge	Adulte, enfant, personnes âgées	isopropanol, chlorure de vinyle, dichlorométhane, perchloroéthylène, tcdd	Clewell *et coll.* 2004
Monte Carlo (Carré Latin)	Adulte	1,1,1-trichloroéthane, trichloréthylène, tetrachloréthylène, benzène, toluène, styrène	Droz *et coll.* 1989a
Monte Carlo (Carré Latin)	Adulte	trichloréthylène, tetrachloréthylène, trichlorure de vinyle, benzène, toluène, styrène	Droz *et coll.* 1989b
Monte Carlo	Adulte	dichlorométhane	Portier et Kaplan 1989
Monte Carlo	Adulte	benzène	Woodruff *et coll.* 1992
Monte Carlo	Adulte	perchloréthylène	Gearhart *et coll.* 1993
Monte Carlo	Adulte	trichloréthylène	Cronin *et coll.* 1995
Monte Carlo	Adulte	trichloréthylène, chlorure de vinyle, chlorure de méthyle	Clewell et Andersen 1996
Monte Carlo	Adulte	benzène	Cox 1996
Monte Carlo	Adulte	benzène, chloroforme, tétrachlorure de carbone, chlorure de méthyle, trichlorure de vinyle, trichloréthylène	Thomas *et coll.* 1996a
Monte Carlo	Adulte	benzène	Thomas *et coll.* 1996b
Monte Carlo	Femmes enceintes (foetus)	méthyle mercure	Clewell *et coll.* 1999
Monte Carlo	Adulte	dichlorométhane	El-Masri *et coll.* 1999
Monte Carlo	Adulte	chloroforme, tétrachlorure de carbone	Delic *et coll.* 2000
Monte Carlo	Adulte	halons	Vinegar *et coll.* 2000
Monte Carlo	Enfants	plomb	Beck *et coll.* 2001
Monte Carlo	Adulte	chlorure de vinyle	Clewell *et coll.* 2001
Monte Carlo	Adulte	éthylène glycol	Sweeney *et coll.* 2001
Monte Carlo	Adulte	warfarin, parathion	Gentry *et coll.* 2002
Monte Carlo	Adulte	*m*-xylène, éthanol	MacDonald *et coll.* 2002
Monte Carlo	Adulte	toluène	Tardif *et coll.* 2002
Monte Carlo	Adulte	chlorpyrifos	Timchalk *et coll.* 2002
Monte Carlo	Adulte	trichloréthylène	Lipscomb *et coll.* 2003
Monte Carlo	Adulte et enfants	dichlorométhane	Pelekis *et coll.* 2003
Monte Carlo	Adulte	*n*-hexane, 2,5-hexanedione	Hamelin *et coll.* 2005

Tableau 1-3 (suite) Exemples d'études de modélisation PBPK sur la variabilité interindividuelle de population.

Approche probabiliste	Population évaluée	Substance	Références
SRMS et Monte Carlo	Adulte	perchloréthylène	Isukapalli *et coll.* 1998
Inférence Bayésienne	Adulte	trichloréthylène	Sohn *et coll.* 2004
MCMC	Adulte	trichloréthylène	Bois 2000
MCMC	Adulte	benzène	Bois *et coll.* 1996
MCMC	Adulte	dichlorométhane	Jonsson *et coll.* 2001a
MCMC	Adulte	chlorure de méthyle	Jonsson *et coll.* 2001b
MCMC	Adulte	dichlorométhane	Jonsson et Johanson 2001a
MCMC	Adulte	toluène	Jonsson et Johanson 2001b
MCMC	Adulte	1,3-butadiene	Smith *et coll.* 2001
MCMC	Adulte	styrène	Jonsson et Johanson 2002

[1]Stochastic Response Surface Methods (SRSM)
[2]Markov Chain Monte Carlo (MCMC)
[3]Références voir bibliographie

Figure 1-1 Facteurs d'incertitude et de variabilité pour le calcul d'une dose de référence (RfC).

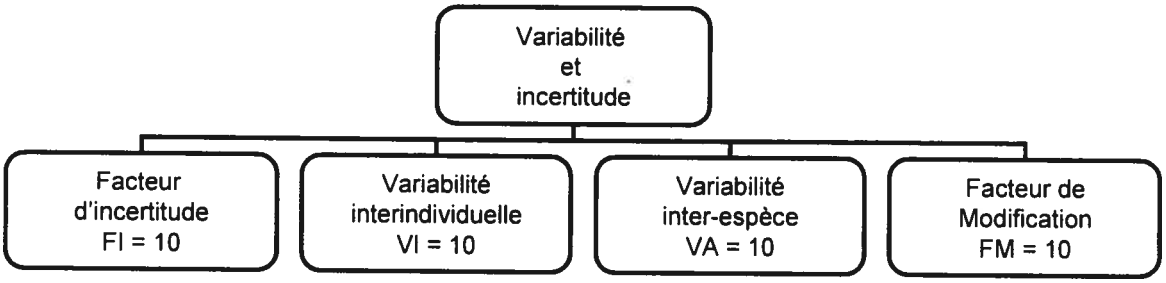
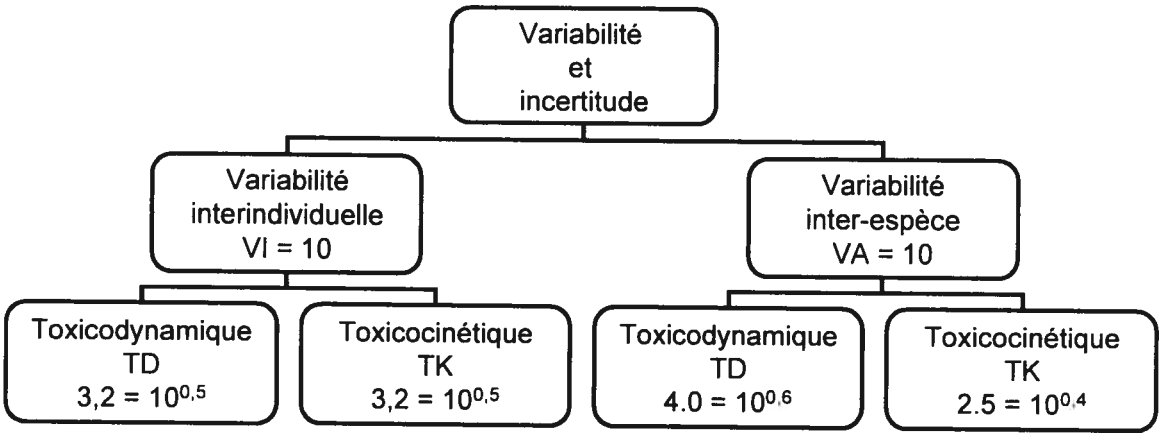


Figure 1-2 Sous division des facteurs de variabilité en composantes toxicodynamique et toxicocinétique.



CHAPITRE II

2. INTRODUCTION À LA MODÉLISATION PHARMACOCINÉTIQUE À BASE PHYSIOLOGIQUE

Mechanistic determinants and modeling of the inhalation pharmacokinetics of volatile organic chemicals.

Andy Nong et Kannan Krishnan.

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Mechanistic determinants and modeling of the inhalation pharmacokinetics of volatile organic chemicals

Andy Nong and Kannan Krishnan.

Groupe de recherche en toxicologie humaine (TOXHUM)
Faculté de médecine, Université de Montréal,
Case Postale 6128, Succursale Centre-ville
Montréal, P.Q., Canada, H3C 3J7

2.1 INTRODUCTION

Pharmacokinetics involves the study of the rate and extent of absorption, distribution, metabolism and excretion of chemicals in biota (Wagner 1975). Inhalation pharmacokinetics relates to the characterization of the time-course of concentration in tissues, blood or excreta of inhaled chemicals and their metabolites. The pharmacokinetics or the processes of pulmonary uptake, tissue distribution, metabolism, urinary and fecal excretion as well as exhalation together determine the amount of inhaled chemicals available for interaction in the tissues. The adverse response in biota is more closely related to internal dose (e.g., concentration of the toxic chemical in the target tissue) rather than inhalation exposure concentration. In fact, dose-response relationships that often appear complex at the exposure dose level become simpler when expressed on the basis of internal dose of the chemical (Gehring et al. 1978, Andersen et al. 1987, Clewell et al. 2002). Figure 2-1 shows an example of refinement of the dose-response relationship for a volatile organic chemical (VOC) resulting from the use of internal dose. Panel A depicts the relationship between the exposure concentration and the observed cancer response for a VOC, which is neither clearly linear nor nonlinear. However, once the exposure concentration is related to the internal dose (e.g., area under the parent chemical concentration vs time of target tissue), the non-linearity due to pharmacokinetics is clearly evident (Panel B). Consequently, the relationship between the internal dose measures and adverse responses can be established more confidently (Panel C). One of the major advantages of constructing dose-response relationships on the basis of internal or delivered doses is that it provides a scientifically-sound basis for conducting extrapolations and for comparing responses observed in various species, exposure concentrations and scenarios (Clewell and Andersen 1994, Medinsky 1995). Physiologically-based pharmacokinetic models are increasingly being used to simulate the internal dose of inhaled VOCs on the basis of mechanistic determinants of uptake and disposition (Ramsey and Andersen 1984, Andersen 1995, 2003, Filser et al. 1995, Leung and Paustenbach 1995, Andersen and Dennison 2001, Krishnan and Andersen 2001).

The internal dose, a key determinant of adverse tissue responses induced by systemically-acting inhaled chemicals, is the net result of pharmacokinetics or the processes of absorption, distribution, metabolism and excretion. This chapter provides an overview of the mechanistic determinants influencing the internal dose and pharmacokinetics of inhaled chemicals, as well as scientifically-sound models for their integration to provide predictions of the pharmacokinetics of inhaled VOCs.

2.2 MECHANISTIC DETERMINANTS OF INHALATION PHARMACOKINETICS OF VOCs

The kinetics of uptake, distribution, metabolism and excretion are determined by a number of factors. These factors are either related to characteristics of the exposed organism (e.g., breathing rate, cardiac output, tissue volumes, tissue blood flow rates, tissue content of metabolizing enzymes) or of the inhaled VOC (e.g., blood: air partition coefficient, tissue: blood partition coefficients, metabolic rate constants). The relative importance of these determinants as they contribute to an increase or a decrease in the internal dose of VOCs, may vary according to the chemical and exposure situations. The general features of these determinants of inhalation pharmacokinetics as well as common methods of assessing them for VOCs are provided in the following paragraphs.

2.2.1 Physiological determinants

Physiological determinants of the inhalation pharmacokinetics of VOCs include: alveolar ventilation rate, cardiac output, tissue blood flow rates, and tissue volumes (Fiserova-Bergerova 1983). The ventilation rate not only determines the rate of uptake of VOCs but also the exhalation rate. The rate of exhalation or pulmonary clearance is determined by the breathing rate as well as the blood:air partition coefficients of VOCs. The ventilation rate in mammals, in general, scales to body surface or a fractional power of body weight ($BW^{0.7}$) (Fiserova-Bergerova 1995). The cardiac output or the systemic

circulation rate determines the speed at which absorbed chemicals are transported to tissues. The blood circulation and therefore the delivery of chemicals to tissues is faster in smaller animals (e.g., mice) than in larger animals (e.g., humans).

Tissue volumes along with their composition determine the extent to which chemicals are diluted within the organism (i.e., volume of distribution). The larger the tissue volumes, the larger the volumes for distribution of chemicals. In addition, the greater the content of lipids in tissues, the larger the volume of distribution, resulting in lower blood concentrations. The tissue blood flow rates, on the other hand, influence the rate at which a chemical in systemic circulation is delivered to a tissue. In any given species, organs such as liver and kidney have the greatest blood perfusion rate compared to the muscle tissue (Table 2-1). Tissues with greater perfusion rates may attain the maximal level of accumulation for a given exposure situation (i.e., steady-state condition) quickly, depending upon their volumes and solubility characteristics.

Some of the physiological parameters that determine the inhalation pharmacokinetics of VOCs can be measured directly in the animal species of interest, whereas others may have to be inferred on the basis of body weight. For example, breathing rates can be measured with the use of a spirometer, plethysmograph, pneumotachograph, hotwire anemometer, or nonbreathing valves (ICRP 1975, Mauderly 1990). Cardiac output has been determined from dye dilution curves using oximeters (Delp et al. 1991). Compilations of physiological determinants and their relationship to body weight are available for a number of species including humans (e.g., Caster et al. 1956, Arms and Travis 1988, Davies and Morris 1993, Brown et al. 1997).

2.2.2 Physicochemical determinants

The physicochemical determinants such as the blood:air partition coefficients and tissue:blood partition coefficients influence the rate of respiratory uptake and distribution of VOCs. Partition coefficients, in general terms, refer to the relative distributions of chemicals between two phases at equilibrium.

The blood:air partition coefficient is a critical determinant of the pulmonary uptake of VOCs. The absorption of VOCs with a relatively high blood:air partition coefficient is limited by alveolar ventilation rate, whereas that of VOCs with low blood:air partition coefficients is limited by cardiac output (reviewed in Fiserova-Bergerova 1983). The blood:air partition coefficient, as described by Henry's law, is the ratio of solubility of a VOC in blood and air. The blood:air partition coefficients of VOCs have most commonly been determined *in vitro* by vial equilibration technique (Sato and Nakajima 1979b, Fiserova-Bergova and Diaz 1986, Johanson and Dynesius 1988, Gargas et al. 1989). Table 2-2 presents the blood:air partition coefficients as well as n-octanol:air partition coefficients for a number of VOCs. The variability of blood:air partition coefficients among VOCs is not explained solely by the differences in octanol or lipid solubility. Rather, the solubility in water should be accounted for, and in some cases binding to blood proteins needs to be accounted for additionally (Poulin and Krishnan 1996b).

Poulin and Krishnan (1996b) developed the following equation for predicting blood:air partition coefficients (P_b) of VOCs that do not bind significantly to blood proteins:

$$P_b = [P_{o:w} P_{w:a} F_{nl}] + [P_{w:a} F_w] \qquad (2\text{-}1)$$

where $P_{o:w}$ = n-octanol:water or oil:water partition coefficient, $P_{w:a}$ = water:air partition coefficient, F_{nl} = neutral lipid-equivalent components in blood, calculated as the sum of neutral lipid content plus 30% of the phospholipid content (expressed as volume fraction), and F_w = water-equivalent components in blood, calculated as the sum of water content plus 70% of phospholipid content (expressed as volume fraction).

In the above equation, the first term represents the partitioning of VOCs between the blood lipids and air whereas the second term represents the partitioning between blood aqueous component and air. P_b of VOCs, according to the above approach, can be calculated with the knowledge of blood composition data, $P_{o:w}$ and $P_{w:a}$. The data on lipid

and water content of rat and human blood are available in the literature and so are the numerical values of $P_{o:w}$ and $P_{w:a}$ at 37°C for several VOCs (Poulin and Krishnan 1995, 1996a,b). For lipophilic low molecular weight VOCs, binding to blood proteins should be accounted for, particularly for predicting P_b in rodents (Poulin and Krishnan 1996b).

Tissue:blood partition coefficients represent another set of physicochemical characteristics that influence the pharmacokinetics of inhaled VOCs. These determinants represent the equilibrium ratio of concentration of a VOC between the tissues and blood. The tissue:blood partition coefficients of adipose tissue and muscle are more important than those for smaller tissues, as critical determinants of the blood concentration of lipophilic VOCs. The larger the tissue:blood partition coefficients, the greater their affnity for tissues. This, of couse, is further influenced by tissue volumes. For example, the volume of adipose tissue represents about 9% and 19%, respectively, in adult rats and humans (Table 2-1).

The tissue:blood partition coefficients for VOCs have been determined by dividing the tissue:air partition coefficients by the blood:air partition coefficient, determined by vial equilibration method (Sato and Nakajima 1979b, Fiserova-Bergova and Diaz 1986, Gargas et al. 1989, Krishnan and Andersen 2001). Tissue:blood, tissue:air and blood:air partition coefficients can also be determined using empirical or semi-empirical methods based on molecular structure information or physicochemical characteristics (Poulin and Krishnan 1998, 1999, Basak et al. 2002, Payne and Kenny 2002, Beliveau et al. 2003, 2005).

2.2.3 Biochemical determinants

Biochemical processes such as biotransformation, macromolecular binding, and renal clearance are key determinants of the inhalation pharmacokinetics of VOCs. Metabolism and renal clearance contribute to decrease in the concentration of a VOC in systemic circulation whereas tissue binding may contribute to increased retention in the body. These biochemical processes will likely be the rate-limiting step of the kinetics of

poorly cleared VOCs. Using time-course data obtained under *in vivo* or *in vitro* conditions, the rates of biochemical processes can be estimated (Dedrick et al. 1972, 1973, Sato and Nakajima 1979a, Reitz et al. 1996, DeJongh and Blaauber 1997, Lipscomb et al. 1998). A strategy that has often been used, involves analysis of data obtained under *in vivo* conditions where pharmacokinetic behavior of a VOC is related to one or two dominant factors and thereby derive estimates of these parameters.

Closed chamber or gas uptake method has commonly been used for estimation of rates of metabolism of VOCs. This method uses a desiccator-type chamber with recirculating atmosphere for exposing groups of animals to VOCs (Andersen et al. 1980; Filser and Bolt 1981, Dennison et al. 2005). Periodic monitoring of the chamber concentration of VOCs during experimental conditions is performed both in the absence and in the presence of animals, for various starting concentrations. The net difference between these two sets of data is attributed to uptake and metabolism by the animals. Since pulmonary uptake is accounted for by the PBPK models, an optimization of metabolic parameters (the only unknown) is done until adequate fit of model simulations to experimental data on chamber concentrations is obtained (Andersen et al. 1980; Filser and Bolt 1981, Gargas et al. 1986). This method has been used successfully to obtain metabolic rate constants of VOCs that are biotransformed by a single first-order process, a saturable process, or a combination of both (Andersen et al. 1987, Gargas et al. 1990, Krishnan et al. 1992). Any chemical or scenario exhibiting a spontaneous loss in excess of ~2% per hour may not be conducive to the use of gas uptake data for the determination of metabolic rate constants. It is also important to monitor the oxygen concentration, humidity level, chamber pressure as well as breathing rates during gas uptake studies (Johanson and Filser 1992, Dallas et al. 1994, Dennison et al. 2005). Since the gas uptake studies involve whole-body exposures, adsorption to fur and dermal uptake may occur. To determine the contribution of adsorption to fur to VOC uptake during whole-body exposures, the exposed animals should be placed in a clean chamber (following the termination of gas uptake exposures), and the time course of the appearance of the chemical characterized (Gargas and Andersen 1989). If the role of dermal absorption is important, then it should be additionally taken into account in the PBPK model (Krishnan and Andersen 2001).

The gas uptake method is not suitable for estimation of metabolic rates of organic chemicals that (i) have low vapor pressure, (ii) exhibit high chamber loss rates, or (iii) possess high blood:air partition coefficient (>60). In such cases, the metabolic rate constants have been assessed using an alternative method, namely, the exhaled breath chamber method (Gargas and Andersen 1989). In this approach, the animals are administered the chemical, and then the time course of chemical in exhaled air is monitored. The data are then analyzed with a PBPK model that contains all parameters except the metabolic constants. By statistical optimiation of model fit to experimental data, the metabolism rate constants are determined (Gargas and Andersen 1989, Gargas 1990). The pharmacokinetic data collected following conventional, constant whole body exposures may also be analyzed with a PBPK model to estimate the metabolic constants (e.g., Tardif et al. 1993).

2.3 MODELING OF INHALATION PHARMACOKINETICS OF VOCs

The physiological, physicochemical and biochemical determinants, discussed in the previous section, together influence the absorption, distribution, metabolism and elimination of VOCs. The internal dose of VOCs (parent chemical or metabolite concentration in target tissues) is determined essentially by the interplay among these factors. Mathematical models that integrate information on these mechanistic determinants are particularly useful not only in facilitating a better understanding of the inhalation pharmacokinetics of VOCs but also in conducting extrapolations essential for risk assessment purposes (interspecies, high to low exposure concentration and exposure scenario extrapolations). In this regard, physiologically-based pharmacokinetic (PBPK) models are important.

For simulating the inhalation pharmacokinetics of VOCs, PBPK models describe the organism as a set of compartments, each of which is characterized physically,

physiologically, and biochemically (Figure 2-2, Ramsey and Andersen 1984). Each of the compartments represent an individual tissue or a group of tissues with similar characteristics. For example, fat depots such as perirenal, epididymal, and omental fat are frequently grouped and represented as a single "fat" compartment (Figure 2-2). If necessary, a "fat" compartment may be subdivided into two or more groups according to the perfusion rates (e.g., inner and subcutaneous adipose tissues). Another example of lumped compartment is the "richly perfused tissues" which consists of adrenal glands, kidney, thyroid, brain, lung, heart, testis, and hepatoportal system. Tissues with poor blood perfusion characteristics (muscle, skin) – that have fairly similar concentrations vs time profiles - are grouped as "poorly perfused tissues". Since the skeletal and structural components of the body have only negligible perfusion and do not contribute significantly to the pharmcokinetic behavior of VOCs, they have not been routinely included in the PBPK models (Krishnan and Andersen 2001).

In the PBPK model illustrated in Figure 2-2, the input results from the inspiration of VOC in the inhaled air at a flow rate equal to the alveolar ventilation rate. The VOC in alveolar air is assumed to be in equilibrium with arterial blood which flows at a rate equal to the cardiac output, and distributes the chemical to liver, fat, richly perfused tissue, and poorly perfused tissue compartments as a function of tissue-specific perfusion rates. The tissue uptake of VOCs appears to be frequently limited by tissue perfusion and therefore has been described according to Fick's law (Table 2-3). Chemicals in venous effluents of the various tissue compartments contribute to the mixed venous concentration that returns to the lung compartment at a flow rate equal to cardiac output (Ramsey and Andersen 1984). Examples of equations constituting PBPK models of VOCs are listed in Table 2-3.

With knowledge of the various input parameters (exposure concentration and duration, physiological parameters, partition coefficients, and metabolic constants), the equations of PBPK models can be solved to provide predictions of the kinetic behavior of chemicals in the test species. Simulation requires solving the set of mass balance differential equations simultaneously by numerical methods. Several commercially-

available simulation or programming software packages can be used for conducting PBPK model simulations (Menzel et al. 1987, Johanson and Naslund 1988, Easterling et al. 2000).

Table 2-4 presents a list of VOCs for which PBPK models have been developed. Current practices for PBPK modeling of various VOCs can be found in Reddy et al. (2005). While most of the PBPK models for VOCs have been validated in rodents, some models have also been extrapolated to humans and validated with limited human pharmacokinetic data. An example of such an effort is illustrated in Figure 2-3. This Figure represents PBPK model simulations of the inhalation pharmacokinetics of toluene and *m*-xylene in human volunteers exposed to 17 ppm and 33 ppm for 7 hr (Tardif et al. 1995).

One of the advantages of PBPK models relates to their use in the conduct of extrapolations on the basis of equivalent internal dose metrics. For example, duration-specific inhalation concentrations of a VOC that would give same internal dose metrics can be simulated using PBPK models. Figure 2-4 depicts an example of 7-hr to 24-hr extrapolation for toluene in humans based on equivalent 24-hr area under the curve (AUC = 0.88 mg/L×hr). These simulations suggest humans exposed to 17 ppm for 7 hr would receive the same internal dose as the humans exposed to 5.38 ppm for 24 hr. These kinds of simulations would not only contribute to refine/reduce animal use in experiments but also help in the design of experiments.

In the PBPK model for toluene described above, pulmonary uptake is represented by assuming that the entire chemical disappearing from the inspired air appears in the arterial blood and that the chemical in alveolar air and arterial blood are in instantaneous equilibrium. Airways such as nasal passages, larynx, trachea, bronchi, and bronchioles are considered as inert tubes that carry the chemical to the pulmonary region, where diffusion occurs. There is evidence that the simple continuous ventilation equilibration models do not adequately predict either total respiratory uptake or regional uptake of highly soluble polar solvents and these solvents appear to have complex relationships between uptake and the blood-air partition coefficient (Johnason 1991). The lower

pulmonary uptake of polar solvents is, in part, due to their adsorption and/or dissolution in the surface of the respiratory epithelium during inhalation and their desorption during exhalation. This adsorption-desorption mechanism is a consequence of both the aqueous solubility of the chemicals and the cyclic nature of respiratory exchange.

PBPK models for polar solvents, in addition to accounting for the anatophysiological characteristics of the respiratory tract, blood flow rates, and partition coefficients of the chemical do take into account the adsorption of vapors during inhalation and desorption during exhalation. The PBPK model for polar solvents developed by Johanson (1991) consists of nine compartments each one representing an anatomical level of the respiratory tree, with the last compartment corresponding to the gas exchange region of the respiratory tract (respiratory bronchioles, alveolar ducts, and alveoli). Each of the first eight compartments is linked with a peripheral compartment facilitating the description of radial diffusion of solvent from the outermost layer and deeper portions of the airway wall. The peripheral compartment of the ninth region corresponds to the rest of body, represented as a single or a multicompartmental PBPK model (Johanson 1991).

The major advantage of PBPK models is their usefulness in predicting tissue dosimetry of chemicals for untested exposure scenarios in laboratory animals, and possibly in humans as well. The validated PBPK models have been used to predict the tissue dose of the potential toxic moiety of VOCs for various exposure concentrations, scenarios and species. This is particularly important since health risk assessments are frequently based on toxicology studies conducted in laboratory animals exposed to high concentrations of VOCs often by scenarios different from anticipated human exposures.

2.4 PREDICTION AND EXTRAPOLATION OF INHALATION PHARMACOKINETICS OF VOCs

PBPK models are uniquely useful in facilitating the conduct of extrapolations of inhalation pharmacokinetics and tissue dosimetry of VOCs from high to low exposure concentrations, species to species and one exposure scenario to another (Clewell and Andersen 1994). These extrapolations are conducted on the basis of internal dose simulations (e.g., area under the parent chemical vs time curve, amount metabolized per unit time). The following sections highlight the use of PBPK models in high dose to low dose and interspecies extapolations of the inhalation pharmacokinetics of VOCs.

2.4.1 High dose to low dose extrapolation

At the high concentrations of VOCs to which animals are exposed during toxicology studies, the internal dose and adverse responses may not be directly proportional to the exposure concentration and could be a result of potentially complex, nonlinear pharmacokinetic processes occurring in the organism at such exposure concentrations (Clewell and Andersen 1984, Andersen et al. 1987). A scientifically-sound extrapolation of internal dose in such cases can be achieved by using PBPK models. These models facilitate the extrapolation of internal dose from high exposure concentration to low exposure concentrations by accounting for the dose-dependency of relevant processes (Clewell and Andersen 1987, D'Souza et al. 1988, Krishnan et al. 1992, Tardif et al. 1995). The description of hepatic metabolism in PBPK models has often included a capacity-limited process, characterized by a maximal velocity and a Michaelis constant, that facilitates simulation of saturation at high exposure concentrations. As such, for the conduct of high dose to low dose extrapolation, no change in the parameters of PBPK model is required, with the exception of the exposure concentration.

The use of PBPK models in the conduct of high dose to low dose extrapolation is exemplified in Figure 2-5. Here, the area under the blood concentration vs time curve (AUC) and the total amount metabolized as a function of exposure concentrations of toluene are presented. These simulation results are indicative of the nonlinear behavior

of toluene in this range of exposure concentrations (1 to 10000 ppm). In such instances, the use of models based on the qualitative and quantitative information on the mechanism of non-linearity should be sought for conducting extrapolation of tissue dose from high exposure concentration to low exposure concentration.

2.4.2 Interspecies Extrapolation

The tissue dosimetry associated with a given exposure concentration of a VOC may differ between species. The interspecies differences in tissue dosimetry may be due to qualitative or quantitative differences in absorption, distribution, metabolism or excretion (ADME) processes. Accounting for the interspecies differences in the occurrence, the magnitude and rate of ADME processes or their parameters allows the prediction of the impact on tissue dosimetry across species. The usefulness of PBPK models for this purpose has been demonstrated with a number of VOCs (Andersen et al. 1991, Reitz et al. 1996, Tardif et al. 1997, Clewell et al. 2002). The procedure intially involves the development of an inhalation PBPK in the test animal species, and then scaling of the physiological parameters of the model as well as replacement of chemical-specific parameters for the species of interest (e.g., humans).

For conducting rat to human extrapolation of inhalation pharmacokinetics of VOCs, quantitative estimates of chemical-specific parameter values (i.e., partition coefficients and metabolic rate constants) in humans are required. The tissue-air partition coefficients of VOCs appear to be fairly constant across species, whereas blood-air partition coefficients show some species-dependency (Gargas et al. 1989, Poulin and Krishnan 1996c). Therefore, the tissue:blood partition coefficients for humans have been calculated by dividing the rodent tissue:air partition coefficients by the human blood:air partition coefficients (Krishnan and Andersen 2001). The species differences in tissue:blood and blood:air partition coefficients for VOCs may also be predicted using species-specific data on the content of neutral lipids, phospholipids, protein and water (Poulin and Krishnan 1995,1996c).

Whereas the adult physiological parameters vary coherently across species, the kinetic constants for metabolizing enzymes do not necessarily follow any type of readily predictable pattern, making the interspecies extrapolation of xenobiotic metabolism rates somewhat difficult. Therefore the metabolic rate constants for VOCs should preferably be obtained in the species of interest. *In vivo* approaches for determining metabolic rate constants are not always feasible or ethical for application in humans. In such cases, metabolism rates in humans may be estimated from *in vitro* data or using a parallelogram approach based on in vivo in rodents and in vitro data obtained using rodent and human tissue microsomal fractions (Reitz et al. 1996, DeJongh and Blaauber 1997, Lipscomb et al. 1998, 2003). In the case of highly metabolized VOCs, most of which are substrates of CYP 2E1, the maximal velocity has been scaled to the $3/4^{th}$ power of body weight, keeping the affinity constant species-invariant. This approach has proved to be an useful approximation of metabolism rates across species particularly in the absence of direct measurements of metabolic rates (e.g., Andersen et al. 1987, Tardif et al. 1997).

Figure 2-6 illustrates the use of PBPK models in the conduct of rat to human extrapolation of the inhalation pharmacokinetics of toluene. In this case, the PBPK model was first developed and validated with inhalation pharmacokinetic data in the test species, i.e., rat (Tardif et al. 1995, 1997). Subsequently, physiological parameters and blood:air partition coefficients for humans were introduced into the model while the metabolic constants were computed on the basis of body weight to the power of 0.7. The extrapolated rat PBPK model adequately predicted the inhalation pharmacokinetics of toluene in human volunteers exposed to 17 ppm for 7 hr (Figure 2-6).

2.5 CONCLUDING REMARKS

The tissue dosimetry and pharmacokinetics of inhaled VOCs are determined by the interplay of several physiological, physicochemical and biochemical factors. PBPK models allow the integration of these factors specific for each chemical and species, thus facilitating the prediction and extrapolation of tissue dosimetry and pharmacokinetics of inhaled VOCs. Using the tissue dose of the toxic moiety of an inhaled toxicant in health

risk assessment calculations provides a better basis than the exposure or atmospheric concentrations of the parent chemical to relate to the observed toxic effects. The development of scientifically-sound approaches for estimation of the pharmacokinetic determinants of VOCs should help *a priori* prediction of their *in vivo* pharmacokinetics in view of helping the design of inhalation toxicology studies and better interpretation of toxicity data resulting from such studies.

2.6 ACKNOWLEDGEMENTS

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2.7 REFERENCES

Andersen, M.E., Development of physiologically based pharmacokinetic and physiologically based pharmacodynamic models for applications in toxicology and risk assessment, *Toxicol. Lett.*, 79, 35-44, 1995.

Andersen, M.E., Toxicokinetic modeling and its applications in chemical risk assessment, *Toxicol. Lett.*, 138, 9-27, 2003.

Andersen, M.E., Clewell, H.J., Gargas, M.L., MacNaughton, M.G., Reitz, R.H., Nolan, R.J., and McKenna, M.J., Physiologically based pharmacokinetic modeling with dichloromethane, its metabolite, carbon monoxide, and blood carboxyhemoglobin in rats and humans, *Toxicol. Appl. Pharmacol.*, 108, 14-27, 1991.

Andersen, M.E., Clewell, H.J. I., Gargas, M.L., Smith, F.A., and Reitz, R.H., Physiologically-based pharmacokinetics and risk assessment process for methylene chloride, *Toxicol. Appl. Pharmacol.*, 87, 185-205, 1987.

Andersen, M.E., and Dennison, J.E., Mode of action and tissue dosimetry in current and future risk assessments, *Sci. Tot. Environ.*, 274, 3-14, 2001.

Andersen, M.E., Gargas, M.L., Jones, R.A., and Jenkins, L.J., Determination of the kinetic constants for metabolism of inhaled toxicants in vivo by gas uptake measurements, *Toxicol. Appl. Pharmacol.*, 54,116, 1980.

Arms, A.D. and Travis, C.C., Reference Physiological Parameters in Pharmacokinetic Modeling. Office of Health and Environmental Assessment, EPA, EPA/600/6-88/004, 1-1-7.16, US Environmental Protection Agency, Washington, DC, 1988.

Basak, S.C., Mills, D., Hawkins, D.M., and El-Marsi, H.A., Prediction of tissue-air partition coefficients: A comparison of structure-based and property-based methods, *SAR QSAR Environ. Res.*, 13, 649-665, 2002.

Beliveau, M., Lipscomb, J., Tardif, R., and Krishnan, K., Quantitative structure-property relationships for interspecies extrapolation of the inhalation pharmacokinetics of organic chemicals, *Chem. Res. Toxicol.*, 18,475-85, 2005.

Beliveau, M., Tardif, R., and Krishnan, K., Quantitative structure-property relationships for physiologically based pharmacokinetic modeling of volatile organic chemicals in rats. J. Toxicol. Appl. Pharmacology., 189, 221-232, 2003.

Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., and Beliles, R.P., Physiological parameter values for physiologically based pharmacokinetic models, *Toxicol. Ind. Health*, 13, 407-484, 1997.

Caster, W.O., Poncelet, J., Simon, A.B., and Armstrong, W.B., Tissue weights of the rat. I. Normal values determined by dissection and chemical methods, *Proc. Soc. Exp. Biol. Med.*, 91, 122-126, 1956.

Clewell, H.J. and Andersen, M.E., Physiologically-based pharmacokinetic modeling and bioactivation of xenobiotics, *Toxicol. Ind. Health*, 10, 1-24, 1994.

Clewell, H.J. and Andersen, M.E., Dose species and route extrapolations using physiologically-based pharmacokinetic modeling, *Drinking Water and Health*, 8,159-184, 1987.

Clewell, H.J., Andersen, M.E., and Barton, H.A., A consistent approach for the application of pharmacokinetic modeling in cancer and noncancer risk assessment, *Environ. Health Perspect.*, 110, 85-93, 2002.

D'Souza, R.W., Francis, W.R., and Andersen, M.W., Physiological model for tissue glutathione depletion and increased resynthesis after ethylene dichloride exposures, *J. Pharmacol. Exp. Ther.*, 245,563-568, 1988.

Dallas, C.E., Muralidhara, S., Chen, X.M., Ramanthan, R., Varkonyl, P., Gallo, J M., and Bruckner, J.V., Use of a physiologically based model to predict systemic uptake and respiratory elimination of perchloroethylene, *Toxicol. Appl. Pharmacol.*, 128, 60-68, 1994.

Davies, B., and Morris, T., Physiological parameters in laboratory animals and humans, *Pharm. Res.*, 10, 1093-1095, 1993.

DeJongh, J., and Blaauboer, B.J., Evaluation of in vitro-based simulations of toluene uptake and metabolism in rats, *Toxicol. in Vitro*, 11, 485-489, 1997.

Dennison, J.E., Andersen, M.E., and Yang, R.S., Pitfalls and related improvements of in vivo gas uptake pharmacokinetic experimental systems, *Inhal. Toxicol.*, 17,539-48, 2005.

Dedrick, R.L., Forrester, D.D., and Ho, D.H.W., In vitro-in vivo correlation of drug metabolism: Deamination of 1-β-D-arabinosyl cytosine, *Biochem. Pharmacol.*, 21, 1-16, 1972.

Dedrick, R.L., Zaharko, D.S., and Lutz, R.J. Transport and binding of methotrexate in vivo, *J. Pharm. Sci.*, 62, 882-890, 1973.

Delp, M.D., Manning, R.O., Bruckner, J.V., and Armstrong, R.B., Distribution of cardiac output during diurnal changes in activity in rats, *Am. J. Physiol.*, 261, H1487-1493, 1991.

Easterling, M.R., Evans, M.V., and Kenyon, E.M., Comparative analysis of software for physiologically based pharmacokinetic modeling: simulation, optimization, and sensitivity analysis, *Toxicol. Meth.*, 10, 203-229, 2000.

Filser, J.G., and Bolt, H.M., Inhalation pharmacokinetics based on gas uptake studies. I. Improvement of kinetic models, *Arch. Toxicol.*, 47, 279-292, 1981.

Filser, J.G., Csanady, G.A., Kreuzer, P.E., and Kessler, W., Toxicokinetic models for volatile industrial chemicals and reactive metabolites, *Toxicol. Lett.*, 82/83, 357-366, 1995.

Fiserova-Bergerova, V., *Modeling of inhalation exopsure to vapors: uptake, distribution and elimination*, Vol. 1, CRC Press Inc., Boca Raton, FL, pp.73-100, 1983.

Fiserova-Bergerova, V., Extrapolation of physiological parameters for physiologically based simulation models, *Toxicol. Lett.*, 79, 77-86, 1995.

Fiserova-Bergerova, V. and Diaz, M.L., Determination and prediction of tissue-gas partition coefficients. Int Arch Occup Environ Health 58, 75-87, 1986.

Gargas, M.L., An exhaled breath chamber system for assessing rates of metabolism and rates of gastrointestinal absorption with volatile chemicals, *J. Am. Coll. Toxicol.*, 9, 447-453, 1990.

Gargas, M.L, and Andersen, M.E., Determinating the kinetic constants of chlorinated ethane metabolism in the rat from rates of exhalation, *Toxicol. Appl. Pharmacol.*, 99, 344-353, 1989.

Gargas, M.L, Andersen, M.E., and Clewell, H.J., A physiologically-based simulation approach for determining metabolic rate constants from gas uptake data, *Toxicol. Appl. Pharmacol.*, 86, 341-352, 1986.

Gargas, M.L, Burgess, R.J, Voisard, D.E, Cason, G.H, and Andersen, M.E., Partition coefficients of low molecular weight volatile chemicals in various liquids and tissues, *Toxicol. Appl. Pharmacol.*, 98, 87-99, 1989.

Gargas, M.L., Clewell, H.J., and Andersen, M.E., Gas uptake inhalation techniques and the rates of metabolism of chloromethanes, chloroethanes and chloroethylenes in the rat, *Inhal. Toxicol.*, 2, 319, 1990.

Gehring, P.J., Watanabe, P.G., Park, C.N., Resolution of dose-response toxicity data for chemicals requiring metabolic activation: example--vinyl chloride, . *Toxicol Appl Pharmacol.*, 44, 581-91, 1978.

International Commission on Radiation Protection (ICRP), *International Commission on Radiation Protection: Report of the task group on reference man.* Pergamon Press, New York, 1975.

Johanson, G., Modelling of respiratory exchange of polar solvents, *Ann. Occup. Hyg.*, 35, 323-339, 1991.

Johanson, G. and Dynesius, B., Liquid: air partition coefficients for six commonly used glycol ethers, *Br. J. Ind. Med.*, 45, 561-564, 1988.

Johanson, G., and Filser, J.G., Experimental data from closed chamber gas uptake studies in rodents suggest lower uptake rate of chemical than calculated from literature values on alveolar ventilation, *Arch. Toxicol.*, 66, 291-295, 1992.

Johanson, G., and Naslund, P.H., Spreadsheet programming: A new approach in physiologically based modeling of solvent toxicokinetics, *Toxicol. Lett.*, 41, 115-127, 1988.

Krishnan, K., and Andersen, M.E. Physiologically based pharmacokinetic modeling in toxicology. In *Principles and methods of toxicology* (A.W. Hayes, Ed.), Taylor & Francis, Philadelphia, PA, pp. 193-241, 2001.

Krishnan, K., Gargas, M.L., Fennell, T.R., and Andersen, M E., A physiologically-based description of ethylene oxide dosimetry in the rat, *Toxicol. Ind. Health*, 8, 121-140, 1992.

Leung, H.W., and Paustenbach, D.J., Physiologically based pharmacokinetic and pharmacodynamic modeling in health risk assessment and characterization of hazardous substances, *Toxicol. Lett.*, 79, 55-65, 1995.

Lipscomb, J.C., Fisher, J.W., Confer, P.D., and Byczkowski, J.Z., In vitro to in vivo extrapolation for trichloroethylene metabolism in humans, *Toxicol. Appl. Pharmacol.*, 152, 376-387, 1998.

Lipscomb, J.C., Teuschler, L.K., Swartout, J., Popken, D., Cox, T., and Kedderis, G.L., The impact of cytochrome P450 2E1-dependent metabolic variance on a risk-relevant pharmacokinetic outcome in humans, *Risk Anal.*, 6, 1221-1238, 2003.

Mauderly, J.L., Measurement of respiration and respiratory responses during inhalation exposures, *J. Am. Coll. Toxicol.*, 9, 397-406, 1990.

Medinsky, M.A., The application of physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling to understanding the mechanism of action of hazardous substances, *Toxicol. Lett.*, 79, 185-191, 1995.

Menzel, D.B., Wolpert, R.L., Boger, J.R., and Kootsey, J.M., Resources available for simulation in toxicology: specialized computers, generalized software and communication networks, *Drinking Water and Health,* 8, 229-254, 1987.

Payne, M.P. and Kenny, L.C., Comparison of models for the estimation of biological partition coefficients, *J. Toxicol. Environ. Health A.* 65,897-931, 2002.

Poulin, P. and Krishnan, K., A biologically-based algorithm for predicting human tissue: blood partition coefficients of organic chemicals, *Hum. Exp. Toxicol.*, 14, 273-280, 1995.

Poulin, P., and Krishnan, K., A tissue composition-based algorithm for predicting tissue: air partition coefficients of organic chemicals, *Toxicol. Appl. Pharmacol.*, 136, 126-130, 1996a.

Poulin, P., and Krishnan, K., A mechanistic algorithm for predicting blood: air partition coefficients of organic chemicals with the consideration of reversible binding in hemoglobin, *Toxicol. Appl. Pharmacol.*, 136, 131-137, 1996b.

Poulin, P. and Krishnan, K., Molecular structure based prediction of the partition coefficients of organic chemicals for physiological pharmacokinetic models, *Toxicol. Methods.*, 6, 117-137, 1996c.

Poulin, P., and Krishnan, K., A quantitative structure-toxicokinetic relationship model for highly metabolised chemicals, *Altern. Lab. Anim.*, 26, 45-59, 1998.

Poulin, P., and Krishnan, K., Molecular structure-based prediction of the toxicokinetics of inhaled vapors in humans, *Int. J. Toxicol.*, 18, 7-18, 1999.

Ramsey, J.C. and Andersen, M.E., A physiologically-based description of the inhalation pharmacokinetics of styrene in rats and humans, *Toxicol. Appl. Pharmacol.*, 73, 159-175, 1984.

Reddy, M., Yang, R.S., Andersen, M.E., and Clewell, H.J., *Physiological based pharmacokinetic modeling: science and applications*, John Wiley & Sons, Inc., Hoboken, NJ, 2005.

Reitz, R.H., Gargas, M.L., Mendrala, A.L., and Schumann, A.M., In vivo and in vitro studies of perchloroethylene metabolism for physiologically based pharmacokinetic modeling in rats, mice, and humans, *Toxicol. Appl. Pharmacol.*, 136, 289-306, 1996.

Tardif R, Lapare S, Krishnan K, Brodeur J., Physiologically based modeling of the toxicokinetic interaction between toluene and m-xylene in the rat, *Toxicol. Appl. Pharmacol.*, 120, 266-73, 1993.

Tardif, R., Lapare, S., Charest-Tardif, G., Brodeur, J., and Krishnan, K., Physiologically-based pharmacokinetic modeling of a mixture of toluene and xylene in humans, *Risk Anal.* 15, 335-342, 1995.

Tardif, R., Charest-Tardif, G., Brodeur, J., and Krishnan, K., Physiologically based pharmacokinetic modeling of a ternary mixture of alkyl benzenes in rats and humans, *Toxicol. Appl. Pharmacol.*, 144, 120-134, 1997.

Sato, A. and Nakajima, T., A vial equilibration method to evaluate the drug metabolizing enzyme activity for volatile hydrocarbons, *Toxicol. Appl. Pharmacol.*, 47, 41-46, 1979.

Sato, A. and Nakajima, T., Partition coefficients of some aromatic hydrocarbons and ketones in water, blood and oil, *Br. J. Ind. Med.*, 36, 231-234, 1979.

Wagner J.G., *Fundamentals of Clinical Pharmacokinetics*, Drug International. Hamilton, IL, 1975.

Table 2-1. Reference physiological parameters for mice, rats, and humans

Physiological parameters	Mouse	Rat	Human
Cardiac output (L/hr)	1.02	4.98	372
Alveolar ventilation (L/hr)	1.5	7.02	300
Tissue perfusions (L/hr)			
Liver	0.258	1.248	97
Fat	0.09	0.45	18.6
Organs	0.522	2.538	164
Muscle	0.156	0.75	93
Tissue volumes (L)			
Liver	0.0014	0.01	2
Fat	0.0025	0.0175	13
Organs	0.0013	0.0125	4
Muscle	0.0175	0.1875	43

Based on Arms and Travis (1988) using typical body weights of 0.025, 0.25 and 70 kg for mouse, rat and human, respectively.

Table 2-2. Blood:air and n-octanol:air partition coefficients of some VOCs (Gargas et al. 1989, Sato and Nakjima 1979a, 1979b)

Chemical	Partition coefficient			
	Blood:Air		Octanol:air	
Vinyl chloride	1.16	±0.08	24.4	±3.7
Vinyl bromide	2.27	±0.16	56	±1.5
Methyl chloride	2.48	±0.23	8.57	±0.22
Carbon tetrachloride	2.73	±0.23	374	±11
Chloroform	6.85	±0.51	402	±12
Trichloroethylene	8.11	±0.17	553	±46
Benzene	8.19	±0.10	465	±5
Tetrachloroethylene	10.3	±1.10	2134	±159
Toluene	15.6	±1.70	1471	±69
m-Xylene	32.5	±1.60	3245	±116
Styrene	51.9	±2.00	5465	±219

Table 2-3. Examples of equations used in inhalation PBPK models for VOCs[1].

Compartment	Equations
Arterial blood	$Ca = \frac{Qc \times Cv + Qp \times Cinh}{Qc + \frac{Qp}{Pb}}$
Liver	$\frac{dC_l}{dt} = \frac{\left(Ql \times (Ca - Cv_l) - \frac{V_{max} \times Cv_l}{Km + Cv_l}\right)}{V_l}$ $Cv_l = \frac{C_l}{P_l}$
Fat	$\frac{dC_f}{dt} = \frac{Qf}{V_f} \times (Ca - Cv_f)$ $Cv_f = \frac{C_f}{P_f}$
Richly perfused tissues	$\frac{dC_r}{dt} = \frac{Qr}{V_r} \times (Ca - Cv_r)$ $Cv_r = \frac{C_r}{P_r}$
Poorly perfused tissues	$\frac{dC_s}{dt} = \frac{Qs}{V_s} \times (Ca - Cv_s)$ $Cv_s = \frac{C_s}{P_s}$
Venous blood	$Cv = \frac{Ql \times Cv_l + Qf \times Cv_f + Qr \times Cv_r + Qs \times Cv_s}{Qc}$
Alveolar air	$Calv = \frac{Ca}{Pb}$

[1]C: Concentration (mg/liter or mmol/liter) , Q: Flow rate (liters.hr^{-1}), V: Volume (liters), P: Tissue:blood partition coefficient, Pb: Blood:air partition coefficient, A Amount (mg), V_{max}: Maximal velocity of enzymatic reaction (mg.hr^{-1}), Km: Michealis Menten affinity constant (mg/L); Subscripts: a = arterial blood, alv = end-alveolar air, f = fat, inh = inhaled air, l = liver, r = richly perfused tissues, s = slowly perfused tissues, v = mixed venous blood, vf = venous blood leaving fat, vl = venous blood leaving liver, vr = venous blood leaving richly perfused tissue, vs = venous blood leaving poorly perfused tissue.

Table 2-4. Volatile organic chemicals for which PBPK models have been developed in one or more species (Krishnan and Andersen 2001).

Chemicals	Species[1]
Acetone	H
Acrylonitrile	R, H
Benzene	R, M, H
Bromochloromethane	R
Bromodichloromethane	R
Bromotrifluoromethane	R
Butadiene (1,3)	R, M, H
Butanol (2-)	R
Carbon tetrachloride	R
Chlorobenzene	H
Chloroethane	R
Chloroform	R, M, H
Chloromethane	R
Cyclohexane	H
Dibromomethane	R
Dichloroethane (1,1-, 1,2-)	R, M
Dichloroethylene (cis, trans)	R
Dioxane (1,4-)	R, M, H
Isopropene	R, M, H
Methanol	R, M, H, Mk
Methyl chloroform	R, M, H
Methyl t-butyl ether	R
Methylene chloride	R, M, H
Methyl ethyl ketone	H
m-Xylene	R, H
n-Hexane	R, H
Tetrachloroethane	R
Tetrachloroethylene	R, M, H, D
Tetrahydrofuran	H
Toluene	R, H
Trichloroethylene	R, M, H
Trifluoroethane	R
Trimethyl benzene	H
Vinyl acetate	R
Vinyl chloride	R
Vinyl fluoride	R

[1]R = rat, M = mouse, H = human, Mk = monkey, D = dog

FIGURE LEGENDS

Figure 2-1. Relationship between the exposure concentration and adverse response for a hypothetical volatile organic chemical (Panel A). Panels B and C represent the relationship between exposure concentration and internal dose (area under the parent chemical concentration vs time curve in target tissue) as well as between internal dose and response.

Figure 2-2. Conceptual representation of a physiologically-based pharmacokinetic model for inhaled VOC. Based on Ramsey and Andersen (1984).

Figure 2-3. Comparison of PBPK model simulations (solid lines) with experimental data on the venous blood concentrations in humans exposed to 17 ppm of toluene (TOL) or 33 ppm of m-xylene (XYL). Based on Tardif et al. (1995).

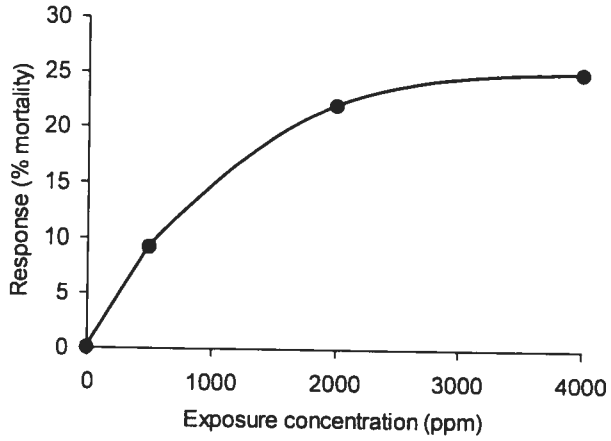
Figure 2-4. PBPK model-derived duration extrapolation (7-hr to 24-hr) of toluene exposures in human based on equivalent 24-hr area under the curve. Panel A displays the simulated blood concentration profile in humans exposed to 17 ppm for 7 hr whereas Panel B depicts the human blood concentration profile associated with 24-hr exposure to 5.38 ppm. Based on Tardif et al. (1997).

Figure 2-5. High dose to low dose extrapolation of internal dose of toluene using a rat PBPK model. (A) Area under the blood concentration vs time curves (AUCs) and (B) total amount metabolized associated with 6-hr inhalation exposure to toluene were calculated for a period of 24-hr. Simulations were based on PBPK model published by Tardif et al. (1997).

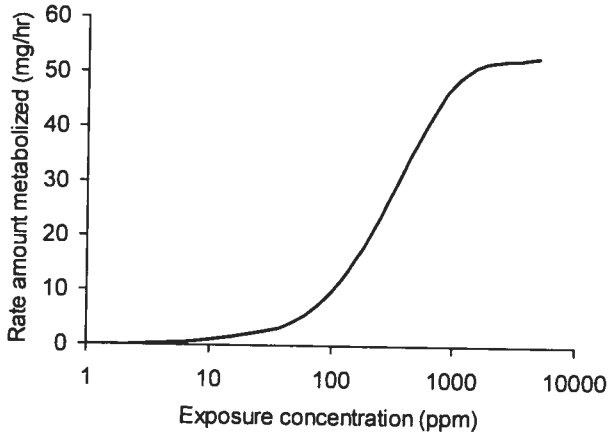
Figure 2-6. Illustration of the use of PBPK models for the conduct of rat to human extrapolation of the inhalation pharmacokinetics of toluene. The simulation in the rat was obtained for 6 hr exposure to 100 ppm of toluene and in human for 7 hr exposure to 17 ppm of toluene. Simulations were obtained using the PBPK model published by Tardif et al. (1997).

Figure 2-1

A



B



C

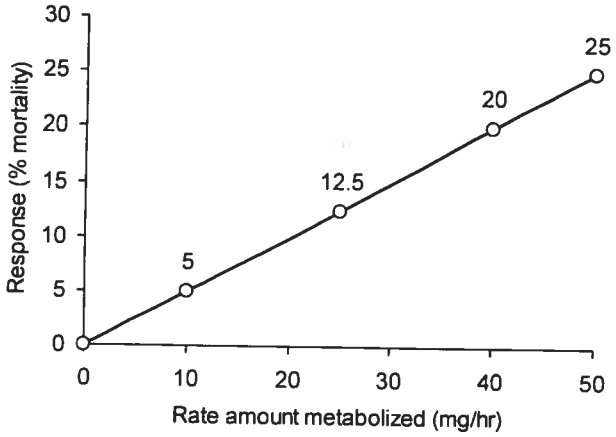


Figure 2-2

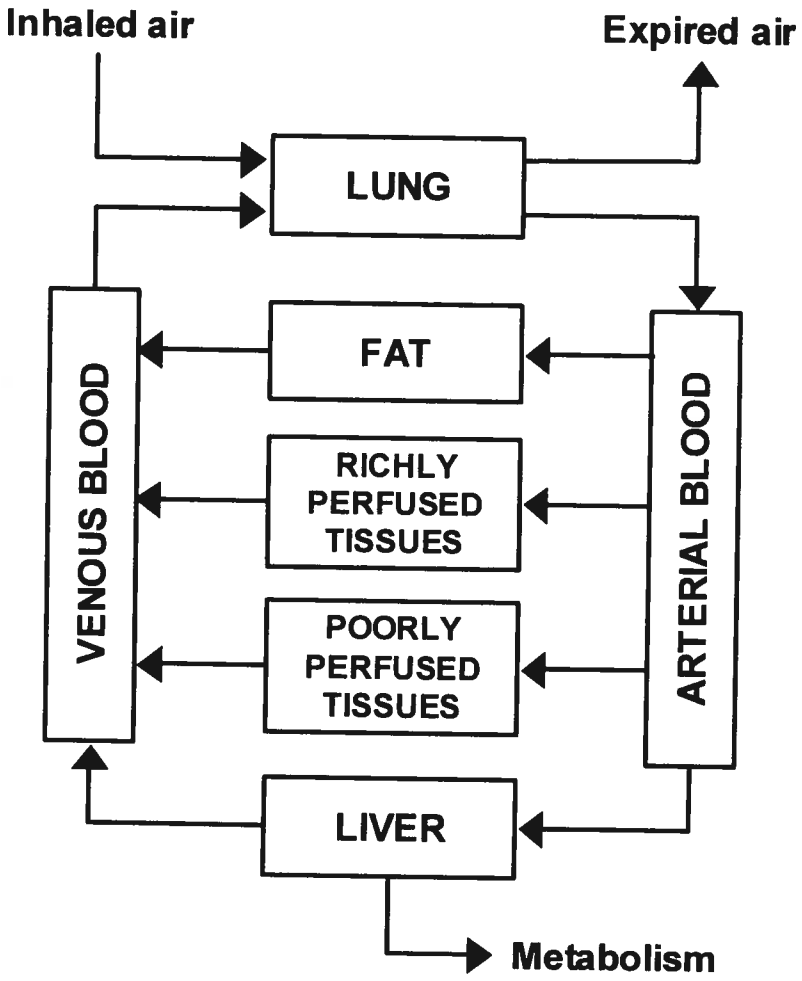

Inhaled air
Expired air
LUNG
FAT
RICHLY PERFUSED TISSUES
POORLY PERFUSED TISSUES
LIVER
VENOUS BLOOD
ARTERIAL BLOOD
Metabolism

Figure 2-3

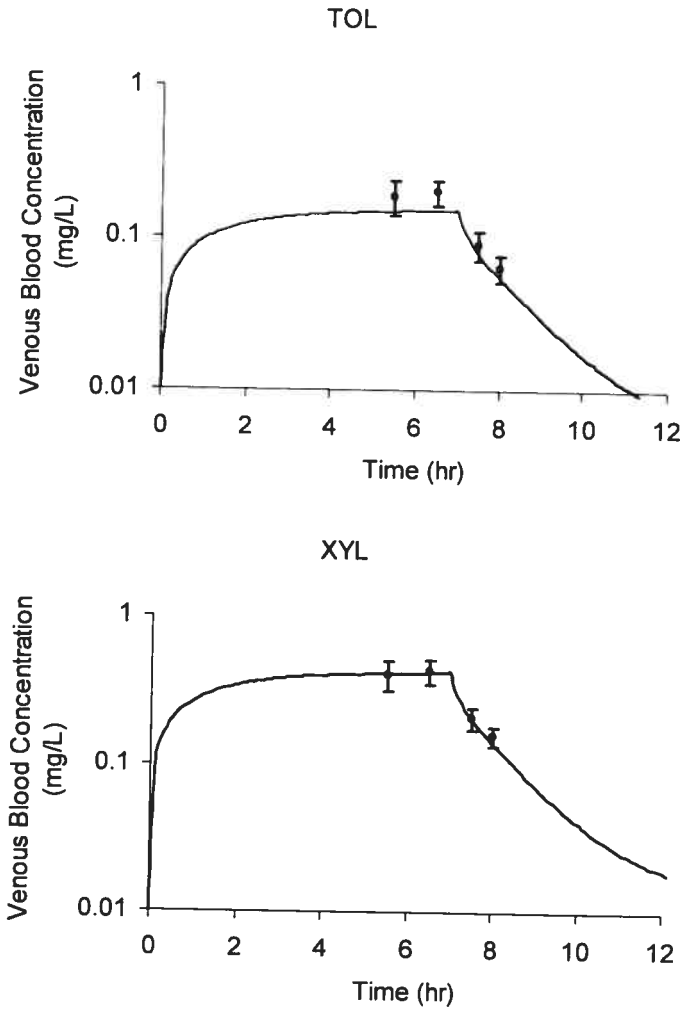

TOL
Venous Blood Concentration (mg/L)
1
0.1
0.01
0
2
4
6
8
10
12
Time (hr)
XYL
Venous Blood Concentration (mg/L)
1
0.1
0.01
0
2
4
6
8
10
12
Time (hr)

Figure 2-4

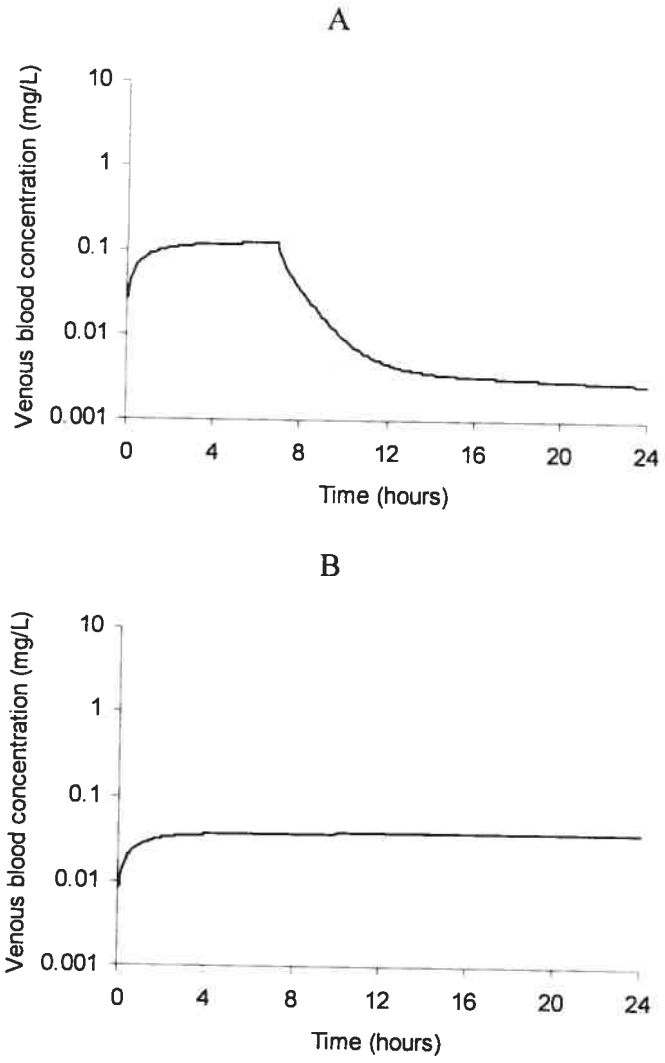
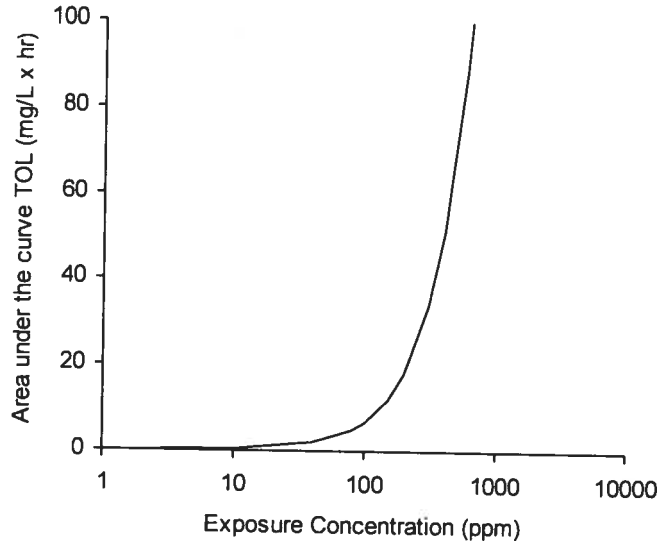

A
Venous blood concentration (mg/L)
10
1
0.1
0.01
0.001
0
4
8
12
16
20
24
Time (hours)
B
Venous blood concentration (mg/L)
10
1
0.1
0.01
0.001
0
4
8
12
16
20
24
Time (hours)

Figure 2-5

A


Area under the curve TOL (mg/L x hr)
100
80
60
40
20
0
1
10
100
1000
10000
Exposure Concentration (ppm)

B

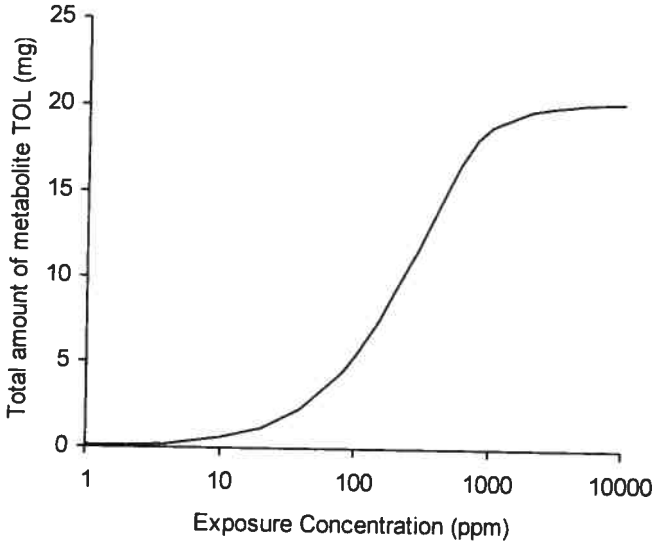
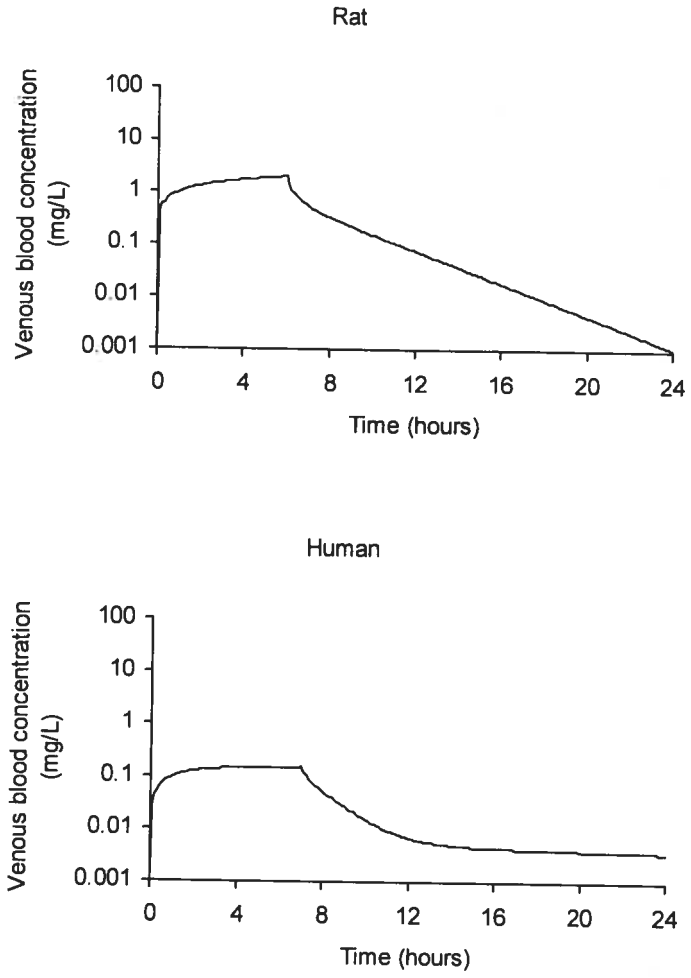

Total amount of metabolite TOL (mg)
25
20
15
10
5
0
1
10
100
1000
10000
Exposure Concentration (ppm)

Figure 2-6


Rat
Venous blood concentration (mg/L)
100
10
1
0.1
0.01
0.001
0
4
8
12
16
20
24
Time (hours)
Human
Venous blood concentration (mg/L)
100
10
1
0.1
0.01
0.001
0
4
8
12
16
20
24
Time (hours)

CHAPITRE III

3. ARTICLE I

Estimation of interindividual pharmacokinetic variability factor for inhaled volatile organic chemicals using a Probability-Bounds approach.

Nong, A. et Krishnan, K.

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Estimation of interindividual pharmacokinetic variability factor for inhaled volatile organic chemicals using a Probability-bounds approach

Andy Nong and Kannan Krishnan

TOXHUM, University of Montreal, 2375 Chemin de la Côte Ste-Catherine, Montreal, Quebec, Canada H3T 1A8

Address for correspondence: K. Krishnan, DSEST, 2375 Cote Ste Catherine Office 4105, University of Montreal, Montreal, PQ, Canada, H3T 1A8.

Email: [redacted], Fax: 514-343-2200.

ABSTRACT

The derivation of reference concentrations (RfCs) for systemically-acting volatile organic chemicals (VOCs) uses a default factor of 10 to account for the interindividual variability in pharmacokinetics (PK) and pharmacodynamics (PD). The magnitude of the PK component of the interindividual variability factor (IVF) has previously been estimated using Monte Carlo approaches and physiologically-based pharmacokinetic (PBPK) models. Since the RfC derivation considers continuous lifetime human exposure to VOCs in the environment, algorithms to compute steady-state internal dose (SS-ID), such as steady-state arterial blood concentration (Ca) and the steady-state rate of amount metabolized (RAM), can be used to derive IVF-PKs. In this context, the probability bounds (P-bounds) approach is potentially useful for computing an interval of probability distribution of SS-IDs from knowledge of population distribution of input parameters. The objective of this study was therefore to compute IVF-PK using the P-bounds approach along with an algorithm for SS-ID in an adult population exposed to VOCs. The existing steady-state algorithms, derived from PBPK models, were re-written such that SS-ID could be related, without any interdependence, to the following input parameters: alveolar ventilation (Qp), hepatic blood flow (Ql), intrinsic clearance (CL_{int}) and blood:air partition coefficient (Pb). The IVF-PK was calculated from the P-bounds of SS-ID corresponding to the 50^{th} and 95^{th} percentiles. Following either specification of probability distribution-free bounds (characterized by minimal, maximal, and mean values) or distribution-defined values (mean, standard deviation and shape of probability distribution where: Qp=normal, Ql=normal, CL_{int}=lognormal, Pb=normal) in RAMAS Risk Calc ® software version 3.0 (Applied Biomathematics, Setauket, NY), the P-bound estimates of SS-ID for benzene, carbon tetrachloride, chloroform and methyl chloroform were obtained for low level exposures (1 ppm). Using only probability distribution-defined inputs, the IVF-PK for benzene, carbon tetrachloride, chloroform and methyl chloroform were respectively 1.18, 1.28, 1.24 and 1.18 (based on P-bounds for Ca), and 1.31, 1.58, 1.30 and 1.24 (based on P-bounds for RAM). A validation of the P-bounds computation was performed by comparing the results with those obtained using traditional Monte Carlo simulation of the steady-state algorithms. In data-poor situations,

when the statistical distributions for all input parameters were not kown or available, the P-bounds approach allowed the estimation of IVF-PK. The use of P-bounds method along with steady-state algorithms, as done in this study for the first time, is a practical and scientifically sound way of computing IVF-PKs for systemically acting VOCs.

3.1. INTRODUCTION

The U.S. EPA approach for deriving inhalation reference concentrations (RfC) applies a 10-fold uncertainty factor (UF) to account for human interindividual variability (U.S. EPA 1994). The internal concentration of inhaled volatile organic chemicals (VOCs) may vary among individuals exposed to the same atmospheric concentration because of biological variability in key determinants (e.g., Clewell *et al.* 2002). The magnitude of the components of the interindividual variability factor, namely, pharmacokinetics (PK) and pharmacodynamics (PD), has been suggested to be 3.2 each (Renwick 1993; Renwick and Lazarus 1998). For environmental pollutants, the magnitude of the PK component of the interindividual variability factor (IVF) may be calculated using physiologically based pharmacokinetic (PBPK) models that make use of the population distribution of mechanistic determinants (Declic *et al* 2000; Jonsson *et al.* 2001; Sweeney *et al.* 2001; Lipscomb *et al.* 2003). Since the RfC derivation considers continuous lifetime human exposure to VOCs in the environment, steady-state algorithms should be sufficient to calculate IVF-PK. Algorithms have been developed to compute steady-state blood and tissue concentrations of VOCs, and these algorithms have been shown to provide identical results as the full-blown PBPK models (Andersen 1981; Pelekis *et al.*, 1997). Nonetheless, these algorithms require fewer parameters than the PBPK models to compute steady-state concentrations.

Even though the derivation of the magnitude of IVF-PK using full-blown probabilistic PBPK models is feasible, it is often limited by the quality and quantity of available data relating to the distributions of all input parameters. In data-poor situations then, the Probability-bounds (P-bounds) method may be used to compute upper and lower bounds of a probability distribution (Ferson 1996; Ferson et al. 1999). The P-bound method computes an area or envelope that is defined by upper and lower bounds at each probability level. The P-bounds for the output (e.g., steady-state blood concentration) are computed using the upper and lower bounds of the input parameters. The parameters can either be described by probability distribution-based bounds (normal, lognormal, uniform, triangular, etc...) or by distribution-free bounds (e.g., minimum, maximum,

mean). The resulting P-bounds correspond to a defined ratio of percentiles or specific cumulative probabilities (Ferson 1996; Ferson et al. 1999). This approach may be applied with the steady-state PK algorithm to quantify IVF-PK in data-poor situations but has never been attempted before.

The objective of this study was to estimate IVF-PK for inhaled VOCs, on the basis of steady-state pharmacokinetic algorithms using the P-bounds approach.

3.2. METHODS

The overall approach involved the use of steady-state PK algorithms along with available information on input parameters in a P-bound method to generate upper and lower bounds of distributions of internal dose measures. More specifically, the P-bounds for steady-state arterial blood concentrations (Ca) as well as steady-state rate of the amount metabolized (RAM) in humans associated with chronic inhalation exposure to VOCs (benzene, carbon tetrachloride, chloroform and methyl chloroform) were generated.

3.2.1. Steady-state algorithms

For steady-state conditions associated with inhalation exposures to VOCs (Figure 3-1), Ca and RAM can be calculated as follows (Andersen 1981; Pelekis *et al.*, 1997; Csanady and Filser 2001):

$$Ca = \frac{Qp \times C_{inh}}{Cl_{pul} + Cl_{hep}} \qquad [3\text{-}1]$$

$$RAM = Cl_{hep} \times Ca \qquad [3\text{-}2]$$

where C_{inh} = inhaled concentration of the chemical, Cl_{pul} = pulmonary clearance and Cl_{hep} = hepatic clearance.

For the P-bounds calculation of Ca and RAM, the above steady-state algorithms were rewritten in order to eliminate the redundancies and interdependence of the parameters. Accordingly,

$$Ca = \frac{Cinh}{\left(\frac{1}{Pb}\right) + \frac{1}{Qp}\left(\frac{1}{\frac{1}{Ql} + \frac{1}{Cl_{int}}}\right)} \qquad [3\text{-}3]$$

$$RAM = \frac{Cinh}{\frac{1}{Pb}\left(\frac{1}{\frac{1}{Ql} + \frac{1}{Cl_{int}}}\right) + \frac{1}{Qp}} \qquad [3\text{-}4]$$

where CL_{int} = intrinsic clearance, Qp = alveolar ventilation rate, Ql = liver blood flow rate and Pb = blood:air partition coefficient.

3.2.2. Input parameters

The population distributions of input parameters, namely, Qp, Ql, CL_{int} and Pb, for benzene, carbon tetrachloride, chloroform and methyl chloroform were obtained from the literature (Table 3-1). The maximal and minimal values of these input parameters were truncated to ± 3 standard deviations (Thomas *et al* 1996).

Specifically, the numerical values and distributions of blood:air partition coefficients correspond to those reported by Thomas *et al.* (1996). The CL_{int} were calculated as the ratio of maximal metabolic velocity (Vmax) and Michealis constant (Km) for each chemical (Rane *et al.* 1977). Using the lognormal distributions of Vmax and Km values from Thomas *et al.* (1996), the distributions of CL_{int} were obtained by using a Monte Carlo approach (10,000 simulations) as seen in Figure 3-2.

The values of Ql and Qp were obtained from a recent compilation of population physiological factors (Price *et al.* 2003) and lognormally distributed as described by Thomas *et al.* (1996).

3.2.3. P-bounds of internal dose and IVF-PK computation

The P-bounds of steady-state arterial blood concentration (Ca) associated with continuous exposure to 1 ppm of benzene, carbon tetrachloride, chloroform and methyl chloroform were computed using RAMAS Risk Calc® software version 3.0 (Applied Biomathematics, Setauket, NY). This program uses semi-analytic algorithms instead of random sampling to determine the upper and lower bounds of probability (P) distributions. The software provides its solutions in the form of the most likely bounds. The parameters with P-defined bounds based on normal or lognormal functions have very narrow range of upper and lower bounds with respect to the discretization error or computational uncertainty of the functions.

The P-bounds of Ca and RAM were computed using the rewritten steady-state algorithms (Eqns. 3-3 & 3-4) with the population characteristics of input parameters. Each parameter was defined with its specific shape, mean, and standard deviation or minmaxmean values. Since the results of the P-bounds are probability intervals (characterized by a minimum and maximum at each percentile), the IVF-PK was calculated using the largest extent or most conservative P-bounds of steady-state internal doses (SS-ID). As seen in the following equation, the magnitude of IVF-PK for each SS-ID was calculated as ratio of the maximal value at the 95th percentile to the minimal value at the 50th percentile:

$$IVF-PK = \frac{[\max imal \cdot 95\% \cdot SS-ID]}{[\min imal \cdot 50\% \cdot SS-ID]} \qquad [5]$$

3.2.4. Validation of P-bounds computation

For the validation of the IVF-PK intervals obtained using the P-bounds method, IVF-PKs were also computed using Monte Carlo simulations with the same steady-state algorithms and parameters. The intervals of confidence of IVF-PK by Monte Carlo simulations were

generated using Crystal Ball® 2000 (Decisioneering, Denver, CO) in Microsoft® Excel. Using the same approach to derive the CL_{int}, the distributed SS-IDs were obtained from 10,000 Monte-Carlo simulations. IVF-PKs were then calculated using the specific percentile values from the distribution of SS-IDs.

3.2.5. Application with minimal data

In data-poor situations, an input parameter may be defined with distribution-free bounds that are based on some known values such as minimum, maximum, and mean (MINMAXMEAN) for purposes of the P-bounds analysis. In the present study, distribution-free bounds or MINMAXMEAN values were applied for the least sensitive parameter (identified based on sensitivity analysis; Table 3-2) to calculate steady-state Ca and RAM. Accordingly, the MINMAXMEAN values for Pb, Qp, Cl_{int}, and Ql respectively for benzene, carbon tetrachloride, chloroform and methyl chloroform were used in computing the P-bounds and IVF-PK.

3.3. RESULTS

Figures 3-3 and 3-4 illustrate the P-bounds of steady-state Ca and RAM, obtained using the mean, standard deviation and shape of the distribution for an adult population as input, for 1 ppm inhalation exposure to benzene, carbon tetrachloride, chloroform and methyl chloroform. Table 3-3 recapitulates the resulting calculations of SS-IDs to derive IVF-PKs using the most descriptive input parameters (shape, mean and standard deviation).

Figure 3-5 compares the P-bounds of Ca (Figure 3-3) with the Monte Carlo simulated distributions. Table 3-4 presents the Monte Carlo simulated SS-IDs to derive IVF-PKs. As seen in Figure 3-5, the Monte Carlo simulated SS-ID distributions lie within the P-bound envelopes. For example, the Monte Carlo computed 50^{th} percentile values (benzene: 17.52, carbon tetrachloride: 15.16, chloroform: 19.56 and methyl chloroform: 13.72 μg/L) are within the P-bounds at that percentile (benzene: [17.39-17.62], carbon

tetrachloride: [15.01-15.23], chloroform: [19.32-19.67] and methyl chloroform: [13.66-13.74]). Similar results for RAM estimates were also obtained with the Monte Carlo and P-bounds approaches (not shown).

Figures 3-6 and 3-7 present the P-bounds of steady-state Ca and RAM for benzene, carbon tetrachloride, chloroform and methyl chloroform using MINMAXMEAN values for the least sensitive input parameters. Table 3-5 summarizes the SS-IDs obtained with this approach and their use in deriving IVF-PKs. Since the distribution-free definition of input parameters contributes to greater variability, the P-bounds in Figures 3-6 and 3-7 are wider than the SS-IDs obtained with information on the shape of distribution for all input parameters (Figure 3-3 and 3-4).

The IVF-PKs based on SS-ID distributions obtained using all three approaches are listed in Table 3-6, and are less than the default factor (3.2) used in risk assessment. The factors estimated in this study range from 1.15 to 1.92, with the larger numbers being associated with the use of MINMAXMEAN values for the least sensitive input parameter. When complete population knowledge of the input parameters is available, the calculated IVF-PK is the comparable between the P-bound approach and the Monte-Carlo method (Table 3-6).

3.4. DISCUSSION

There is increasing interest in approaches that allow the substitution of the default IVF-PK with more appropriate values (Gundert-Remy et al. 2002). The actual values of IVF-PK may be specific to chemicals, toxic endpoints and exposed population. Even though the default IVF-PK factor of 3.2 is generally expected to protect about 95 percent of the population, recent studies have raised some serious concerns that it may not afford adequate protection for some sub-populations such as newborns and elderly (Dourson *et al.* 1996; Dorne *et al.* 2003, 2004). It is unethical and impractical to collect PK information in neonates, children, adults, elderly, pregnant women and lactating mothers

in order to compute the magnitude of IVF-PK for each chemical. A feasible alternative is to use population data on mechanistic determinants in models or algorithms. In this regard, PBPK models have been used to estimate the IVF-PK, mostly for adult populations for some chemicals (e.g., (Declic *et al* 2000; Jonsson *et al.* 2001; Sweeney *et al.* 2001; Lipscomb *et al.* 2003). One of the challenges relates to the availability of the population distribution data for all input parameters of these models. Since IVF-PK are often applied in risk assessments that focus to protect humans in the context of continued lifetime exposures, it would be appropriate to use simple, steady-state algorithms to estimate IVF-PK for VOCs. The main advantage of the use of steady-state algorithm with the P-bounds approach, as done in the present study, is that it captures the critical determinants of interindividual variability of the internal dose during chronic exposure to VOCs. Since the steady-state algorithm relies only upon a few parameters (Qp, Ql, Pb and Cl_{int}), it is rather easy to identify the physiological and determinants that would affect the internal dose metrics in a population.

Several studies using a Monte Carlo approach have calculated IVF-PK as the ratio between the 95^{th} and 50^{th} percentiles of internal dose (Allen *et al.* 1996, Bois *et al.* 1996, Thomas *et al.* 1996, Delic *et al.* 2000). The magnitude of IVF-PK for several VOCs computed in this manner was generally within a factor of 3.2, consistent with the observations of the present study. Here, the reported IVF-PK were obtained using a more conservative method, i.e., the maximal value associated with the 95^{th} percentile and the minimal values associated with 50^{th} percentile. In principle, the true IVF-PK would lie between the most conservative estimation (maximal 95^{th} and minimal 50^{th}) and the smallest difference between the P-bounds (minimal 95^{th} and maximal 50^{th}). The IVF-PK for selected VOCs in the current study using the latter approach would have ranged from 0.06 to 0.98 (not shown). Nonetheless, the range of estimated IVF-PKs from different combinations of 95^{th} and 50^{th} percentile values from the calculated P-bounds would still be less than the default factor for all the VOCs investigated in this study.

The present study uniquely demonstrates the estimation of population variability of internal doses based on the available information. The flexibility of the P-bound method

is that it allows it to compute IVF-PK according to various input values available, even with a minimal data set. Data requirements can be determined using a sensitivity analysis on the input parameters for calculation of SS-IDs, and subsequently the least sensitive parameters can be defined with MINMAXMEAN values along with more complete statistical characterization of sensitive parameters. For instance, when the distributions of parameter values are not definitely known, mean, maximal and minimal values based on available data may be used as input to generate the P-bounds of internal dose metrics for a defined population. The range of IVF-PKs computed with MINMAXMEAN values are comparable to the IVF-PKs calculated with complete population descriptors (mean, standard deviation, and shape of the distribution). As shown in this study, the IVF-PK intervals from the Monte Carlo method are within the intervals computed by the P-bounds approach. Therefore, both probabilistic methods are equivalent in computing an interval of confidence of the IVF-PKs. Since the Monte Carlo method is limited to the size of data and constraints of the mathematical distribution, the P-bounds approach is more practical with partial population data (Ferson 1996). Accordingly, the choice of the method would depend upon the availability of information on the population variability of the input parameters. The Monte Carlo simulation requires a defined statistical knowledge of the parameters, whereas the parameters of the P-bound approach can be established upon well-known statistical inferences such as MINMAXMEAN values.

Even though inhalation is considered as the main route of exposure to VOCs, dermal and ingestion routes of exposure may also be important and can be incorporated into the steady-state algorithms to characterize to facilitate the computation of route-specific magnitude of IVF-PK. The steady-state algorithm combined with P-bounds approach allows the use of a variety of data for the input parameters: (i) distributions for each of the parameters, (ii) MINMAXMEAN for each of the parameters, and (iii) distribution for sensitive parameters combined with MINMAXMEAN for all other parameters. The present study has demonstrated that the IVF-PK obtained with the P-bounds approach are comparable to that of the Monte Carlo simulation approach, when same statistical distributions for each of the input parameters are used in both approaches. However, the P-bounds approach has the added advantage of being able to generate envelopes of

probability distribution of IVF-PK when there is only partial or minimum data for the input parameters of the steady-state algorithm. Overall, the use of steady-state algorithm along with the P-bounds approach should be useful as a tool for estimating population-specific IVF-PK in data-poor situations.

3.4.1. Conclusion

In conclusion, the use of Probabilistic bounds method with steady-state algorithms, for as done in this study for the first time, is a practical and scientifically-sound way of computing the magnitude of IVF-PK for use in the health risk assessment of systemically-acting VOCs.

3.5. REFERENCES

Allen BC, Covington TR, Clewell HJ. 1996. Investigation of the impact of pharmacokinetic variability and uncertainty on risks predicted with a pharmacokinetic model for chloroform. Toxicology. 111:289-303.

Andersen ME. 1981. Pharmacokinetics of inhaled gases and vapors. Neurobehavioral Toxicol and Teratol 3:383-389.

Bois FY, Jackson ET, Pekari K, Smith MT. 1996. Population toxicokinetics of benzene. Environ Health Perspect. 104 Suppl 6:1405-11.

Burmaster, D.E., Llyod, K.J., Thompson, K.M. 1995. The need for new methods to backcalculate soil cleanup targets in interval and probabilistic cancer risk assessments *Hum. Ecol. Risk Assessment.* 1, 89-100.

Clewell HJ, Teeguarden JG, McDonald T, Sarangapani R, Lawrence GS, Covington TR, Gentry R, Shipp A. 2002. Review and evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. Crit Rev Toxicol 32:329-389.

Csanady GA, Filser JG. 2001. The relevance of physical activity for the kinetics of inhaled gaseous substances. Arch Toxicol. 74:663-72.

Delic JI, Lilly PD, MacDonald AJ, Loizou GD. 2000. The utility of PBPK in the safety assessment of chloroform and carbon tetrachloride. Regul Toxicol Pharmacol 32:144-155

Dorne JL, Walton K, Renwick AG. 2003. Human variability in the renal elimination of foreign compounds and renal excretion-related uncertainty factors for risk assessment. Food Chem Toxicol 42:275-298.

Dorne JL, Walton K, Renwick AG. 2004. Human variability for metabolic pathways with limited data (CYP2A6, CYP2C9, CYP2E1, ADH, esterases, glycine and sulphate conjugation). Food Chem Toxicol 42:397-421.

Dourson ML, Felter SP, Robinson D. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. Regul Toxicol Pharmacol. 24:108-20.

Ferson S. 1996. What Monte Carlo Methods cannot do. Hum Ecol Risk Assess 2:990-1007.

Ferson S, Root WT, Kuhn R. 1999. RAMAS Risk Calc: Risk Assessment with Uncertain Numbers. Applied Biomathematics. Setauket, New York.

Gargas ML, Andersen ME. 1988. Physiologically based approaches for examining the pharmacokinetics of inhaled vapors. In: Gardner DE, Crapo JD, Massaro EJ, editors. Toxicology of the lung. New York: Raven Press.p 449-476.

Gundert-Remy U, Sonich-Mullin C; IPCS Uncertainty and Variability Planning Workgroup and Drafting Group (International Program on Chemical Safety's). The use of toxicokinetic and toxicodynamic data in risk assessment: an international perspective. Sci Total Environ. 288:3-11.

Jonsson F, Johanson G. 2003. The Bayesian population approach to physiological toxicokinetic–toxicodynamic models—an example using the MCSim software. Toxicol Let 138:143-150.

Jonsson F, Bois F, Johanson G. 2001. Physiologically based pharmacokinetic modeling of inhalation exposure of humans to dichloromethane during moderate to heavy exercise. Toxicol Sci. 59:209-18.

Lipscomb J.C., Teuschler L.K., Swartout J., Popken D., Cox T., and Kedderis G.L. 2003. The impact of cytochrome P450 2E1-dependent metabolic variance on a risk-relevant pharmacokinetic outcome in humans. Risk Anal. 23, 1221-38.

Pelekis M, Krewski D, Krishnan K. 1997. Physiologically based algebraic expressions for predicting steady-state toxicokinetics of inhaled vapors. Toxicol Meth 7:205-225.

Portier CJ, Kaplan NL. 1989. Variability of safe estimated when using complicated models of carcinogenic processes. A dose study: methylene chloride. Fundam Appl Toxicol 13:533-544.

Price P.S., Conolly R.B., Chaisson C.F., Gross E.A., Young J.S., Mathis E.T., and Tedder D.R. (2003). Modeling interindividual variation in physiological factors used in PBPK models of humans. Crit Rev Toxicol. 33, 469-503.

Renwick AG. 1993. Data-derived safety factors for the evaluation of food additives and environmental contaminants. Food Addit Contam 10:275-305.

Renwick AG, Lazarus NR. 1998. Human variability and non-cancer risk assessment - an analysis of the default uncertainty factor. Regul Toxicol Pharmacol 27:3-20.

Rane A, Wilkinson GR, Shand DG. 1977. Prediction of hepatic extraction ratio from in vitro measurement of intrinsic clearance. J Pharmacol Exp Ther 200:420-424.

Sweeney LM, Tyler TR, Kirman CR, Corley RA, Reitz RH, Paustenbach DJ, Holson JF, Whorton MD, Thompson KM, Gargas ML. 2001. Proposed occupational exposure limits for select ethylene glycol ethers using PBPK models and Monte Carlo simulations. Toxicol Sci 62:124-139.

Thomas RS, Bigelow PL, Keefe TJ, Yang RSH. 1996. Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. Am Ind Hyg Assoc J 57:23-32.

US Environmental Protection Agency (USEPA). 1994. *Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry.* USEPA, Washington, DC, EPA/600/8-90/066F.

Table 3-1 Population characteristics of input parameters

Parameter	Min	Mean	Max	SD	Shape
Alveolar ventilation[1] (L/min)	7.96	11.57	16.57	1.42	Lognormal
Liver blood flow[1] (L/min)	0.79	1.32	2.14	0.22	Lognormal
Intrinsic clearance[2] (L/min)					
Benzene	0.33	1.74	7.07	0.95	Lognormal
Carbon tetrachloride	0.20	1.07	4.39	0.59	Lognormal
Chloroform	2.66	14.32	58.95	7.91	Lognormal
Methyl chloroform	0.006	0.030	0.119	0.016	Lognormal
Blood:air partition coefficient[2]					
Benzene	7.68	8.19	8.70	0.17	Normal
Carbon tetrachloride	1.35	2.73	4.11	0.46	Normal
Chloroform	3.79	6.85	9.91	1.02	Normal
Methyl chloroform	1.75	2.53	3.31	0.26	Normal

[1]Price *et al.* 2003
[2]Thomas *et al.* 1996

Table 3-2 Sensitivity analysis of the parameters of SS-IDs

	Benzene	Carbon Tetrachloride	Chloroform	Methyl Chloroform
Alveolar ventilation	++	+	++	+++
Liver blood flow	+	+	+++	+
Intrinsic clearance	+++	+++	+	++
Partition coefficient	+	+++	+++	+++

Sensitivities of the parameters on the change of SS-BCs are scaled as:
+++ : 20% contribution to the variance
++ : 10 - 20 % contribution to the variance
+ : < 10% contribution to the variance
Parameters with the least sensitivity in the shaded cells were used to compute SS-IDs using minmaxmean values.

Table 3-3 **95^{th} and 50^{th} percentiles of SS-IDs using shape, mean and standard deviation for each input parameter**

	Benzene	Carbon Tetrachloride	Chloroform	Methyl Chloroform
Ca (μg/L)				
Max 50^{th}	17.39	15.01	19.32	13.66
Min 95^{th}	20.52	19.18	23.98	16.09
RAM (μg/min)				
Max 50^{th}	18.32	21.52	18.59	54.94
Min 95^{th}	24.03	33.99	24.24	68.22

Table 3-4 95^{th} and 50^{th} percentiles of SS-IDs based on Monte Carlo simulation

	Benzene	Carbon Tetrachloride	Chloroform	Methyl Chloroform
Ca (μg/L)				
Max 50^{th}	17.52	15.16	19.56	13.72
Min 95^{th}	20.21	18.94	23.64	16.03
RAM (μg/min)				
Max 50^{th}	18.51	21.94	18.84	55.31
Min 95^{th}	23.41	32.62	23.75	67.80

Table 3-5 95^{th} and 50^{th} percentile of SS-IDs using MINMAXMEAN.

	Benzene	Carbon Tetrachloride	Chloroform	Methyl Chloroform
Ca (µg/L)				
Max 50^{th}	16.88	14.47	18.83	13.66
Min 95^{th}	21.17	19.69	26.02	16.09
RAM (µg/min)				
Max 50^{th}	17.93	19.65	17.85	54.91
Min 95^{th}	24.38	37.76	27.40	68.28

Table 3-6 Comparison of IVF-PKs estimated with P-bounds and Monte Carlo simulation approaches

Approach	Benzene	Carbon Tetrachloride	Chloroform	Methyl Chloroform
Ca				
P-defined bounds (shape, mean, SD)	1.18	1.28	1.24	1.18
P-free bounds (minmaxmean)	1.25	1.36	1.38	1.18
Monte Carlo simulated	1.15	1.25	1.21	1.17
RAM				
P-defined bounds (shape, mean, SD)	1.31	1.58	1.30	1.24
P-free bounds (minmaxmean)	1.36	1.92	1.53	1.24
Monte Carlo simulated	1.26	1.49	1.26	1.23

FIGURE LEGENDS

Figure 3-1 Steady-state version of a PBPK model for inhaled volatile organic chemicals. Qp is the pulmonary ventilation, Ql is the hepatic blood flow, Cl_{int} is the intrinsic clearance and Pb is the blood:air partition coefficient.

Figure 3-2 Monte Carlo simulations of intrinsic clearance (Cl_{int} = Vmax/Km) of benzene, carbon tetrachloride, chloroform and methyl chloroform in humans after 10,000 iterations based on values from Thomas et al. (1996).

Figure 3-3 P-bounds of steady state arterial blood concentrations (Ca, μg/L) in an adult population exposed to 1 ppm benzene, carbon tetrachloride, chloroform and methyl chloroform using input parameters characterized with mean, standard deviation, and shape of the distribution.

Figure 3-4 P-bounds of steady state rate of amount metabolized (RAM μg/hr) in an adult population exposed to 1 ppm benzene, carbon tetrachloride, chloroform and methyl chloroform using input parameters characterized with mean, standard deviation, and shape of the distribution.

Figure 3-5 Comparison of the P-bounds (solid lines) and Monte Carlo (dotted lines) simulated distributions of steady state arterial blood concentration (μg/L) for an adult population exposed to 1 ppm benzene, carbon tetrachloride, chloroform and methyl chloroform.

Figure 3-6 P-bounds of steady state arterial blood concentrations (Ca μg/L) for an adult population exposed to 1 ppm benzene, carbon tetrachloride, chloroform and methyl chloroform using MINMAXMEAN values for the least sensitive parameter for each chemical.

Figure 3-7 P-bounds of steady state rate of amount metabolized (RAM μg/hr) for an adult population exposed to 1 ppm benzene, carbon tetrachloride, chloroform and methyl chloroform using MINMAXMEAN values for the least sensitive parameter for each chemical.

Figure 3-1

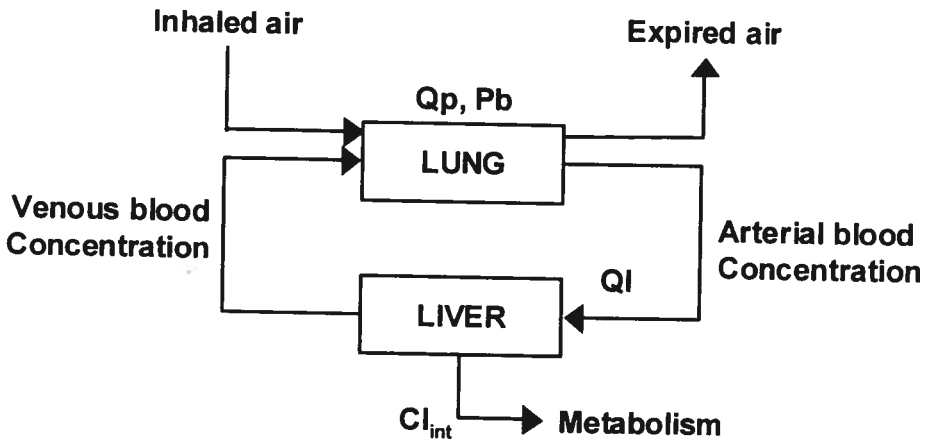

Inhaled air
Expired air
Qp, Pb
LUNG
Venous blood
Concentration
Arterial blood
Concentration
Ql
LIVER
Cl_{int}
Metabolism

Figure 3-2

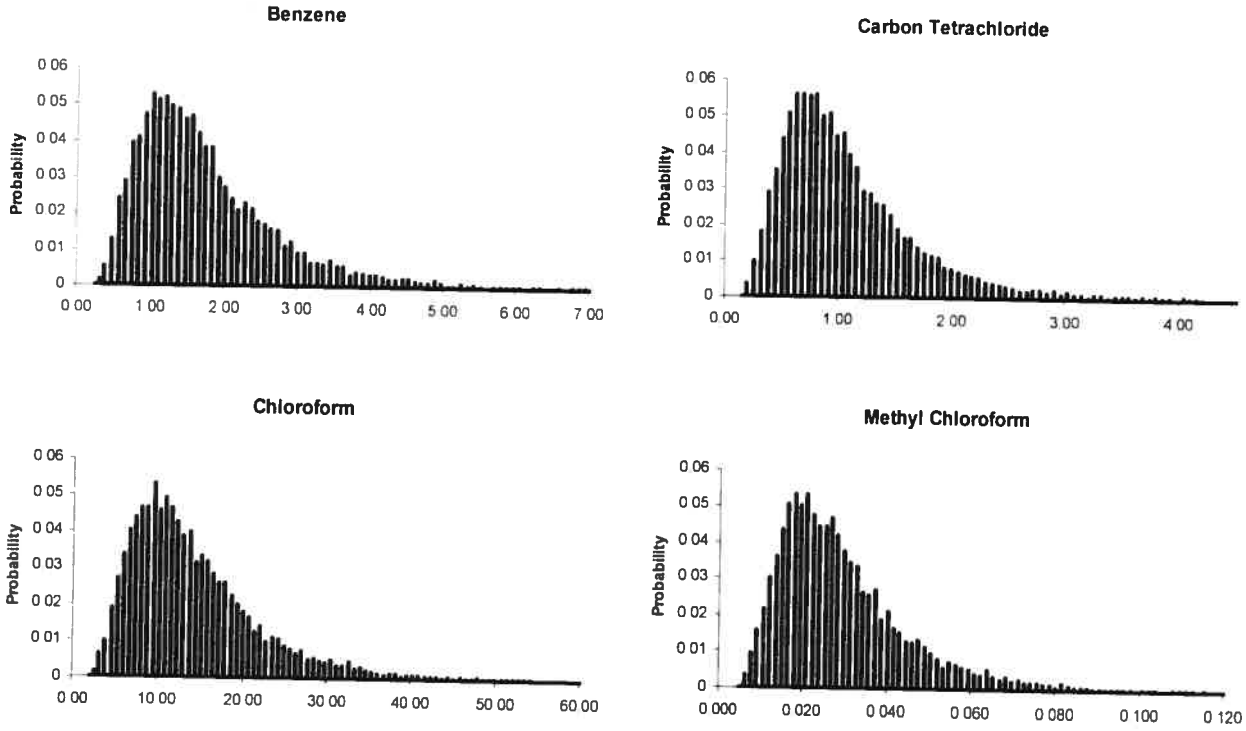

Benzene
Probability
0 06
0 05
0 04
0 03
0 02
0 01
0
0 00
1 00
2 00
3 00
4 00
5 00
6 00
7 00
Carbon Tetrachloride
Probability
0 06
0 05
0 04
0 03
0 02
0 01
0
0 00
1 00
2 00
3 00
4 00
Chloroform
Probability
0 06
0 05
0 04
0 03
0 02
0 01
0
0 00
10 00
20 00
30 00
40 00
50 00
60 00
Methyl Chloroform
Probability
0 06
0 05
0 04
0 03
0 02
0 01
0
0 000
0 020
0 040
0 060
0 080
0 100
0 120

Figure 3-3

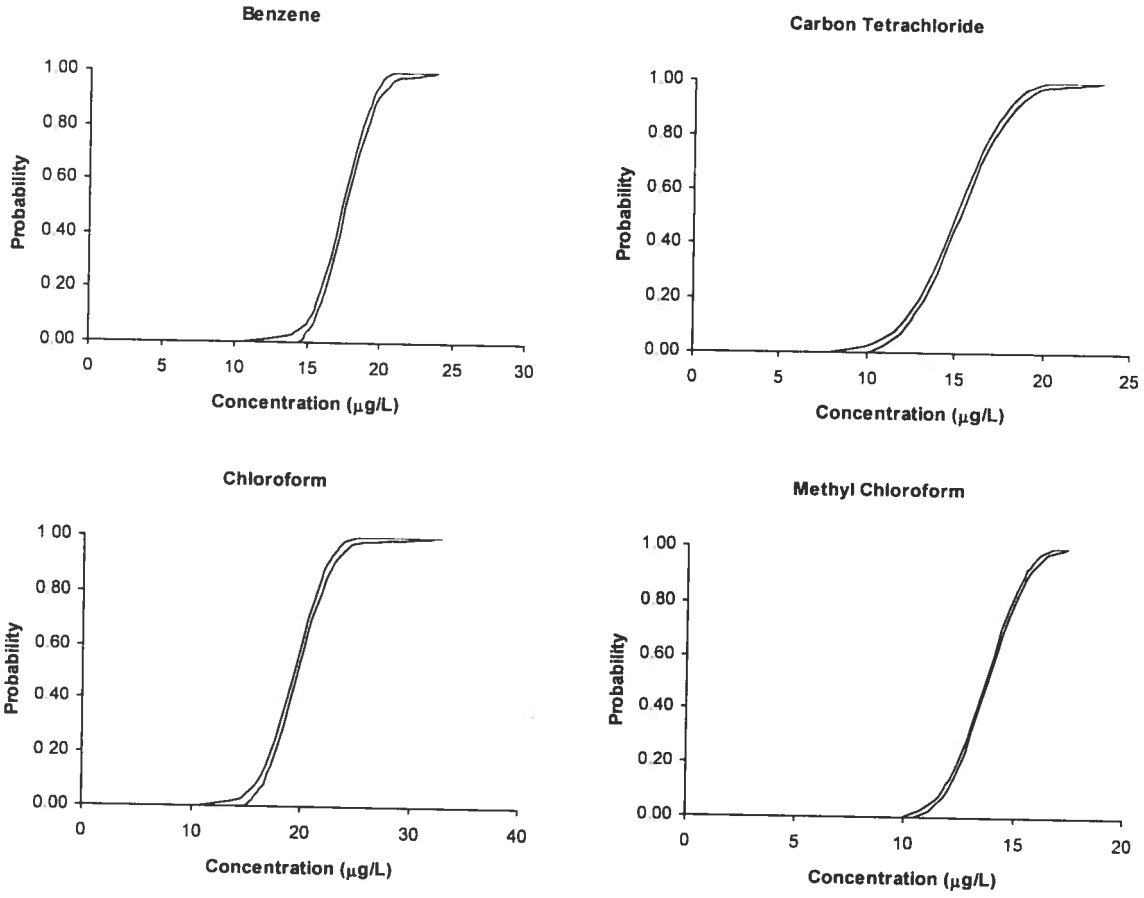

Benzene
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
5
10
15
20
25
30
Concentration (µg/L)
Carbon Tetrachloride
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
5
10
15
20
25
Concentration (µg/L)
Chloroform
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
10
20
30
40
Concentration (µg/L)
Methyl Chloroform
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
5
10
15
20
Concentration (µg/L)

Figure 3-4

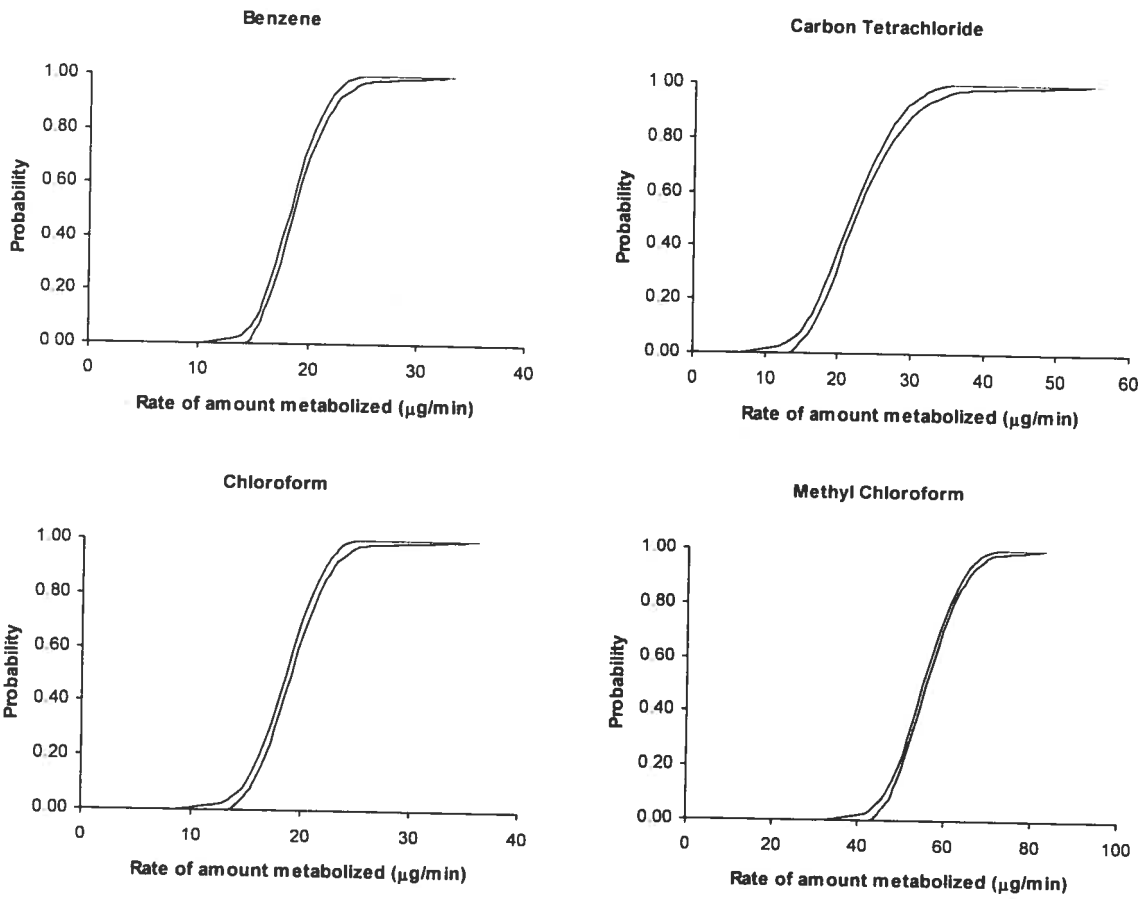

Benzene
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
10
20
30
40
Rate of amount metabolized (µg/min)
Carbon Tetrachloride
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
10
20
30
40
50
60
Rate of amount metabolized (µg/min)
Chloroform
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
10
20
30
40
Rate of amount metabolized (µg/min)
Methyl Chloroform
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
20
40
60
80
100
Rate of amount metabolized (µg/min)

Figure 3-5

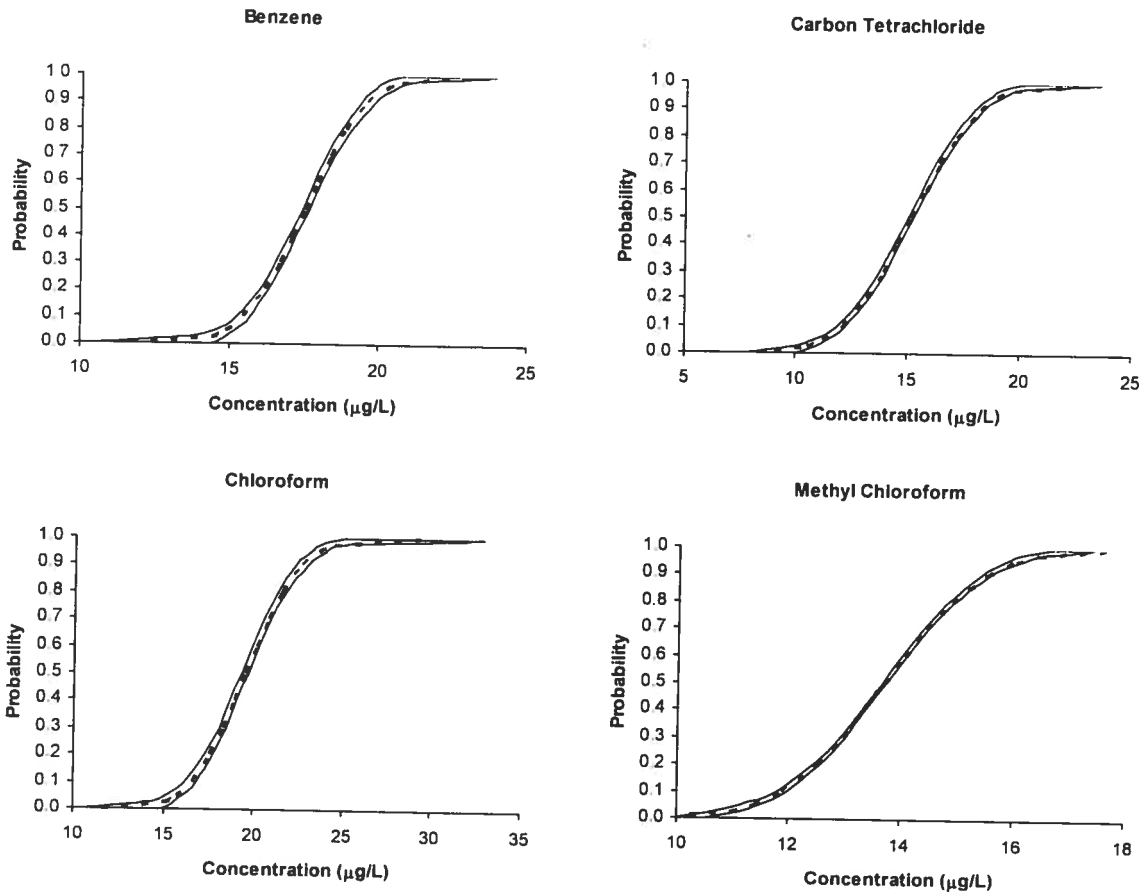

Benzene
Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10
15
20
25
Concentration (µg/L)
Carbon Tetrachloride
Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
5
10
15
20
25
Concentration (µg/L)
Chloroform
Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10
15
20
25
30
35
Concentration (µg/L)
Methyl Chloroform
Probability
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
10
12
14
16
18
Concentration (µg/L)

Figure 3-6

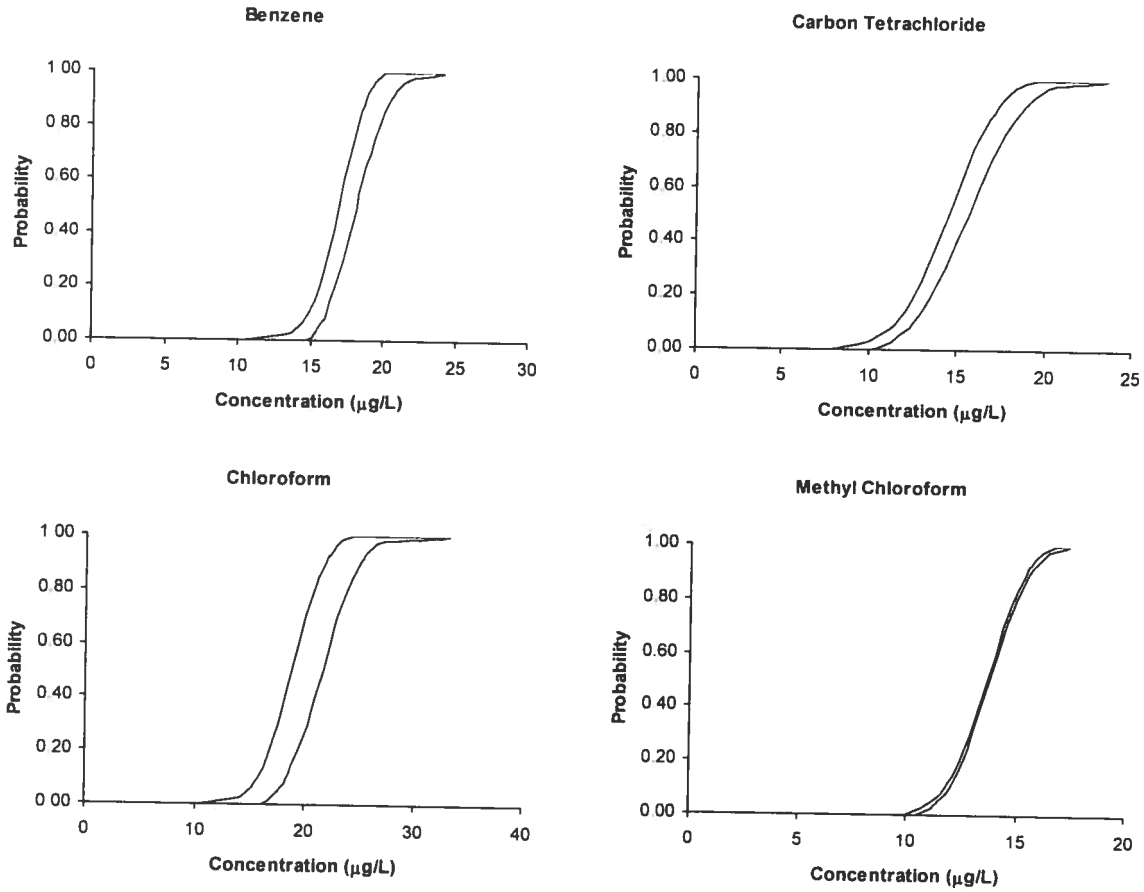
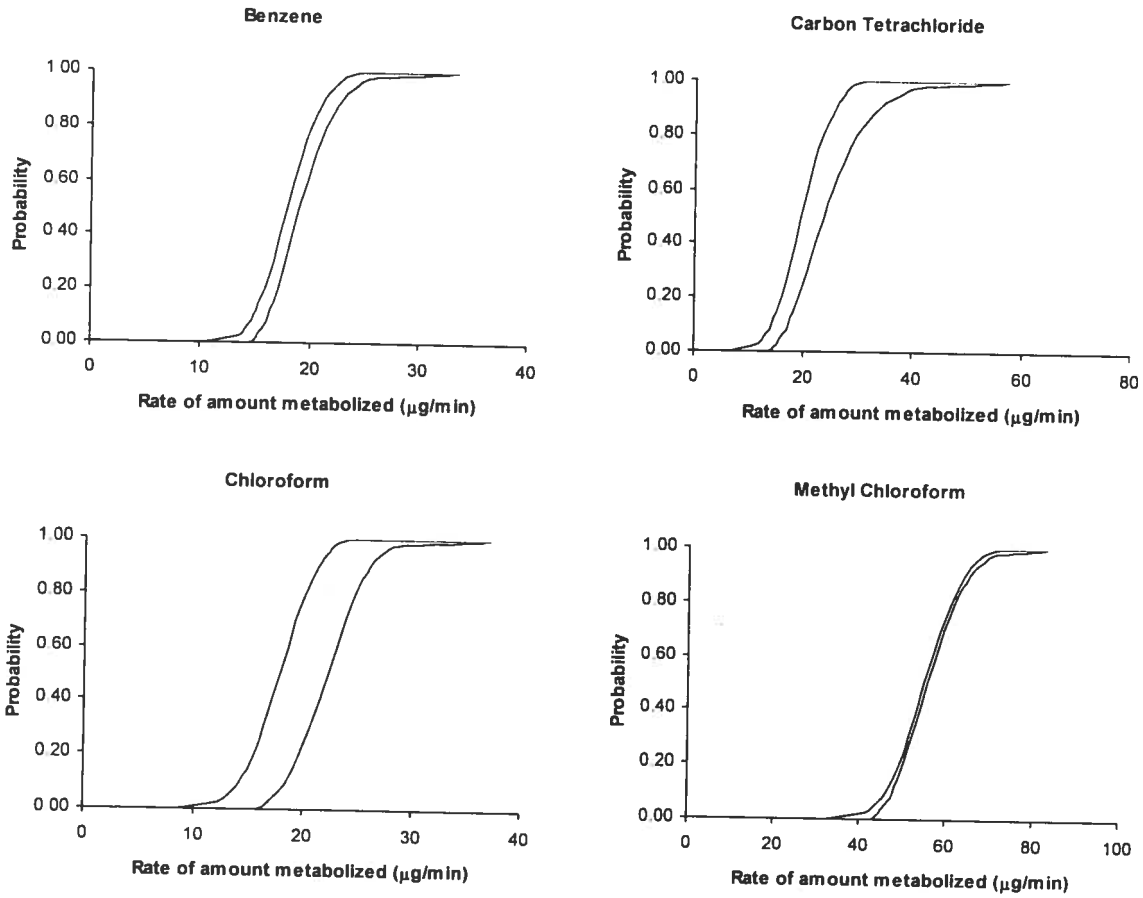

Benzene
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
5
10
15
20
25
30
Concentration (µg/L)
Carbon Tetrachloride
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
5
10
15
20
25
Concentration (µg/L)
Chloroform
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
10
20
30
40
Concentration (µg/L)
Methyl Chloroform
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
5
10
15
20
Concentration (µg/L)

Figure 3-7


Benzene
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
10
20
30
40
Rate of amount metabolized (µg/min)
Carbon Tetrachloride
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
20
40
60
80
Rate of amount metabolized (µg/min)
Chloroform
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
10
20
30
40
Rate of amount metabolized (µg/min)
Methyl Chloroform
Probability
1.00
0.80
0.60
0.40
0.20
0.00
0
20
40
60
80
100
Rate of amount metabolized (µg/min)

CHAPITRE IV

4. ARTICLE II

Modeling inter-child differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: A case study with toluene.

Nong, A., McCarver, D.G., Hines, R.N. et Krishnan, K.

Nong, A, McCarver, D.G., Hines, R.N., and Krishnan K. 2006. Modeling inter-child differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: A case study with toluene. Toxicology Applied Pharmacology. 214, 78-87. http://dx.doi.org/10.1016/j.taap.2005.12.001

Modeling inter-child differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: A case study with toluene

Nong, A[1], McCarver, D G.[2], Hines, RN[2] and Krishnan K[1]

[1]Occupational and Environmental Health, Université de Montréal, Montreal, QC, Canada.
[2]Department of Pediatrics, Medical College of Wisconsin, Milwaukee, WI, USA.

Address for correspondence: K. Krishnan, DSEST, 2375 Cote Ste Catherine Office 4105, University of Montreal, Montreal, PQ, Canada, H3T 1A8. Email: Kannan.Krishnan@umontreal.ca, Fax: 514-343-2200.

ABSTRACT

The objective of the present study was to evaluate the magnitude of inter-individual variability in the internal dose of toluene in children of various age groups, on the basis of subject-specific hepatic CYP2E1 content and physiology. The methodology involved the use of a previously validated physiologically-based pharmacokinetic (PBPK) model, in which the intrinsic clearance for hepatic metabolism (CL_{int}) was expressed in terms of the CYP2E1 content. The adult toluene PBPK model, with enzyme content-normalized CL_{int}, facilitated the calculation of child-specific CL_{int} based on knowledge of hepatic CYP2E1 protein levels. The child-specific physiological parameters, except liver volume, were computed with knowledge of age and body weight, whereas physicochemical parameters for toluene were kept age-invariant based on available data. The actual individual-specific liver volume (autopsy data) was also included in the model. The resulting model was used to simulate the blood concentration profiles in children exposed by inhalation, to 1 ppm toluene for 24 hr. For this exposure scenario, the area under the venous blood concentration *vs* time curve (AUC) ranged from 0.30 to 1.01 μg/ ml×hr in neonates with low CYP2E1 concentration (<3.69 pmol/mg protein). The simulations indicated that neonates with higher levels of CYP2E1 (4.33 to 55.93 pmol/mg protein) as well as older children would have lower AUC (0.16 to 0.43 μg/ml×hr). The latter values were closer to those simulated for adults. Similar results were also obtained for 7 hr exposure to 17 ppm toluene, a scenario previously evaluated in human volunteers. The inter-individual variability factor for each sub-group of children and adults, calculated as the ratio of the 95^{th} and 50^{th} percentile values of AUC, was within a factor of 2. The 95^{th} percentile value of the low metabolizing neonate group, however, was greater than the mean adult AUC by a factor of 3.9. This study demonstrates the feasibility of incorporating subject-specific data on hepatic CYP2E1 content and physiology within PBPK models for evaluating the age, inter-child and population variability of internal dose for use in risk assessment of inhaled volatile organics.

Key words: PBPK model, child, CYP2E1, interindividual variability

4.1. INTRODUCTION

A factor of 10 is conventionally applied in the health risk assessment of volatile organic chemicals (VOCs) to account for the pharmacokinetic (PK) and pharmacodynamic variability among individuals in a population. The PK portion of the interindividual variability factor (IVF-PK) has been suggested to be equal to 3.17 (reviewed in Dorne and Renwick 2005). This default value may not hold true for all chemicals and in such cases chemical-specific IVF-PK can be computed using available data (e.g., IPCS 2001). When population data on the PK of chemicals are not available, the population variability of PK determinants or parameters can be potentially useful in estimating IVF-PK (Dorne *et al.* 2005). Alternatively, the information on population variability of PK determinants may be incorporated within physiology-based models to estimate IVF-PK (Clewell and Andersen 1996; Thomas *et al.* 1996; Kedderis and Lipscomb 2001; Clewell *et al.* 2002; Jonsson and Johanson 2001; Lipscomb *et al.* 2003; Price *et al.* 2003b). Lipscomb *et al.* (2003) estimated IVF-PK by incorporating data on inter-individual variability (n = 60, age = 22-65 years) of CYP2E1, the major isozyme involved in the hepatic metabolism of low molecular weight VOCs (Guengerich *et al.* 1991; Nakajima *et al.* 1991), within physiologically-based PK (PBPK) models. However, no such effort has been made to evaluate the magnitude of inter-child variability of PK as a function of age.

Toluene (TLV: 188 mg/m^3, RfC: 5 mg/m^3) (ACGIH 2005; US EPA 2005), a VOC found in glues, paints and cleaning solvents, has been associated with childhood exposures (e.g., Rumchev *et al.* 2004; Sexton *et al.* 2005). Whereas acute exposure to toluene leads to central nervous system (CNS) effects (Foo *et al.* 1990; US EPA 2005), chronic exposures have been associated with both neurological and respiratory effects (NTP 1990; ATSDR 2000). Toluene in the parent chemical form is thought to be responsible for these toxic effects (Benignus *et al.* 1998, 2005; Haddad *et al.*1999). Animal and human studies have indicated that toluene is metabolized primarily to benzyl alcohol (Nakajima *et al.* 1991; Tassaneeyakul *et al.* 1996; Nakajima *et al.* 1997). *Ortho-* and *para-* cresols represent minor metabolites of toluene metabolism and have been detected in urine of exposed animals and humans (Ogata 1984; Lof *et al.* 1993; Tardif *et al.* 1998).

Studies with human and rat liver microsomes have demonstrated that CYP2E1 is the most active isozyme in forming benzyl alcohol, particularly at low exposure concentrations, with CYP1A2 being active in forming the minor metabolites (cresols) (Nakajima *et al.* 1991; Nakajima and Wang 1994; Tassaneeyakul *et al.* 1996). Given that there is evidence of age-related differences in the expression of hepatic cytochrome P450 enzymes (Sonnier and Cresteil 1998; Johnsrud *et al.* 2003; Stevens *et al.* 2003; Koukouritaki *et al.* 2004), the current study probed whether age-related differences in hepatic CYP2E1 levels and physiology together would contribute substantially to interindividual variability in PK and internal dose of toluene. By constructing PBPK models that contained subject-specific data on CYP2E1 levels and physiology, the variability in the internal dose of toluene in children of various age groups was simulated.

4.2. METHODS

4.2.1. Subject-specific data

The data on subject-specific hepatic CYP2E1 content were obtained from a recent study on the ontogeny of hepatic CYP2E1 expression (Johnsrud *et al.* 2003). This study analyzed autopsy samples from various age groups and developmental stages for hepatic CYP2E1 content. For the current study, data for 116 samples (41 males, 75 females), corresponding to age groups ranging from newborn to 17 years old, were utilized. For each of these subjects, in addition to age, body weight (Figure 4-1A), liver volume (Figure 4-1B) and the data on CYP2E1 protein content (pmol/mg microsomal protein) (Figures 4-1C-D) were obtained. The raw data presented in Figure 1 can be obtained by writing to the corresponding author (kannan.krishnan@umontreal.ca).

4.2.2. Child PBPK model

The incorporation of child-specific data on CYP2E1 levels along with other relevant physiological parameters was accomplished using a previously validated adult PBPK model for toluene (Tardif *et al.* 1995). A subject-specific inhalation PBPK model for toluene (Figure 4-2) was developed for children belonging to various age groups, by using physiological parameters and metabolism rate specific to each individual child.

The body weight and liver volume for each child were obtained from autopsy record. The tissue blood flow rates for children weighing between 4 kg and 50 kg were computed using subject-specific body weight information according to Price *et al.* (2003a) who published quantitative relationships among body weight, age and physiological parameters (i.e., alveolar ventilation rate, cardiac output, and blood flow rate to tissues). The tissue volumes (except liver volumes) were calculated according to age-specific body weights and tissue volumes (fat, muscle and skin, and skeleton) based on Haddad *et al.* (2001) as shown in Figure 4-3. The volume of the rest of the body compartment was determined as the difference between body weight and the sum total of the volumes of liver, fat, muscle, skin, and skeleton. The fraction of cardiac output flowing through various tissues and the fraction of body weight represented by the various tissue compartments, calculated for a 4-kg child were used as the basis for calculating the parameter values for children of lower body weights (< 4 kg). The hepatic blood flow rates for children less than 4 kg were computed in relation to an average neonate portal vein flow of 100 ml/min (Kao *et al.* 1996). Similarly, these parameters available for a reference adult were used as the basis for computing the flows and volumes of children weighing over 50 kg (Haddad *et al.* 2001).

The chemical-specific input parameters, namely intrinsic clearance and partition coefficients, for toluene were obtained from Tardif *et al.* (1995). The partition coefficients (blood:air and tissue:blood) were considered age-invariant since the available data do not indicate significant variation in the lipid and water contents of most tissues and blood as a function of age (Cahalan *et al.* 1981; White *et al.* 1991; Pierce *et al.* 1996; Price *et al.* 2003a).

The intrinsic clearance of toluene in each child ($CL_{int\text{-}child}$) was calculated as follows:

$$CL_{int-child} = \left(\frac{CL_{int-adult}}{[CYP2E1]_{adult} \times V_{liver-adult}} \right) \times [CYP2E1]_{child} \times V_{liver-child} \qquad [4\text{-}1]$$

Based on adult intrinsic clearance ($CL_{int\text{-}adult}$, L/hr) normalized to CYP2E1 content (pmol CYP2E1/mg protein) and volume of adult liver (L), $CL_{int\text{-}child}$ was calculated with the knowledge of hepatic CYP2E1 level and liver volume in each child. Accordingly, intrinsic clearance of toluene in adults was initially obtained as V_{max}/Km (V_{max} = 116.16 mg/hr for a 70 kg person, Km = 0.55 mg/L; Tardif *et al.* 1995). The $CL_{int\text{-}adult}$ was then divided by the average CYP2E1 content of adult liver (48.9 pmol/mg microsomal protein in Lipscomb *et al.* 2004) as well as the liver volume (1.82 L for an individual weighing 70 kg). The child-specific intrinsic clearance $CL_{int\text{-}child}$ (L/hr) was then computed using the subject-specific hepatic CYP2E1 protein content ($[CYP2E1]_{child}$ in pmol/mg protein) and liver volume ($V_{liver\text{-}child}$ in L) . The child-specific intrinsic clearance values as a function of age, computed in this study, are depicted in Figure 4-4 (A: neonates; B: older children). In the present study, adult-children difference in microsomal protein, if any, was not taken into account, due to lack of data. In other words, the present study assumed that the microsomal protein content of adult and infant livers is comparable and the impact of this assumption may be verified as relevant data become available.

The hepatic clearance (CL_h) of toluene in PBPK model of each child was computed as follows (Wilkinson and Shand 1975; Poulin and Krishnan 1999):

$$CL_h = \frac{CL_{int} \times Ql}{CL_{int} + Ql} \qquad [4\text{-}2]$$

where Ql = liver blood flow rate (L/hr).

Equations 4-1 & 4-2 together permit to account for the age-specific CYP2E1 content, liver weight and hepatic blood flow rate in computing the rate of metabolism within the PBPK model (Poulin and Krishnan 1999).

4.2.3. PBPK model simulations

The PK profile of inhaled toluene in each individual child was simulated using a PBPK model containing subject-specific information on CYP2E1 content as well as

physiological data. The mean and range of parameter values used in the modeling exercise are given in Tables 4-1 & 4-2. The PBPK model was written in MS Excel® (Haddad *et al.* 1996), and simulations of toluene kinetics following 7 hr exposure to 17 ppm (a-third of the TLV; ACGIH 2005) and 24 hr exposure to 1 ppm were conducted. The former exposure concentration and duration correspond to those of a previous study in which adult volunteers were exposed to toluene for collection of data on blood concentrations (Tardif *et al.* 1997), whereas the latter exposure concentration and duration were used to simulate a scenario relevant for a risk assessment (i.e., exposure concentration equivalent to the rounded value of RfC; US EPA 2005). Since no PK data on toluene are available in children, the intent was at least to compare the simulations of the child-specific PBPK model with the experimental data previously collected in adult volunteers (17 ppm, 7 hr; Tardif *et al.* 1997).

4.2.4. Statistical methods

Probability density frequencies (PDFs) associated with internal dose surrogates (peak concentration (Cmax), area under the venous blood concentration *vs* time curve (AUC) over a 24-hour period) were computed for various subgroups of children as well as for adults. The PDF values obtained for each subgroup were plotted as a function of the internal dose metrics to characterize the distribution. The magnitude of intra-group variability was computed as the ratio between the 95th and 50th percentile values for each group (i.e., neonate, infant, child, adolescent and adult). The IVF-PK was then calculated as the ratio between the 95th percentile value for each sub-group and 50th percentile value for the adult population (IPCS 2001; Dourson *et al.* 2002). The population distribution of internal dose metrics for adults was computed by Monte Carlo simulation approach (10,000 simulations) using Microsoft Excel® and Crystal Ball® (version 5.5, Decisioneering, Denver, CO). The PBPK model structure and input values along with their distributions were obtained from Tardif *et al.* (1995) and Thomas *et al.* (1996) (Tables 4-1 & 4-2).

Sensitivity analysis of the PBPK model parameters, including physiological and hepatic CYP2E1 content, was conducted for each age group and sensitivity ratios (reflecting

percent change in AUC associated with 5% variation in the value of input parameters) were calculated (US EPA 2001). Sensitivity of hepatic clearance of toluene (Eqn. 2) was also assessed in order to identify the relative importance of blood flow and CYP2E1 content in children of various age groups.

4.3. RESULTS

4.3.1. PBPK model simulations

The PBPK model simulations of venous blood concentration profiles of toluene in children exposed to 17 ppm for 7 hr are presented in Figure 4-5. The simulated PK profiles are compared with the experimental data on venous blood concentrations collected in adult human volunteers following a similar exposure scenario (Tardif *et al.* 1997). In general, the adult values are within the envelope of PK profiles simulated by the child-specific PBPK models (Figure 4-5). The peak venous blood concentration (corresponding to the value associated with the end of exposure) in 28 neonates ranged from 0.21 to 0.70 μg/ml whereas that for other groups of children ranged from 0.11 to 0.29 μg/ml (Figure 4-6). The relationship between Cmax and age depicted in Figure 4-6 shows that there is greater inter-subject variability in neonates under a month old compared to older children. As observed in Figure 4-6A, about half the neonate group exhibited higher Cmax (0.51 to 0.70 μg/ml; low metabolizers) compared to the rest (0.21 to 0.45 μg/ml; high metabolizers). The neonates exhibiting low Cmax are also the ones with lower hepatic clearance (Figure 4-7A). Given that the blood flow rate is comparable between the two groups of neonates, the low hepatic extraction in low metabolizers (Figure 4-7B) is a consequence of low CYP2E1 levels.

Figure 4-8 shows that the 24-hour area under the venous blood concentration vs time curves (AUCs) for 17 ppm exposure range from 1.70 to 5.78 μg/ml×hr in neonates and from 0.91 to 2.62 μg/ml×hr in all other children. Figure 4-8A shows that the 28 neonates (< 1 month old) fall under two categories, one with higher AUC values (4.58 to 5.78 μg/ml×hr) and the other with lower AUC values (1.70 to 3.61 μg/ml×hr) comparable to older children (0.91 to 2.62 μg/ml×hr) (Figure 4-8B). The model simulations are

consistent with the trend that neonates under the age of one month with low hepatic CYP2E1 content (<3.69 pmol/mg microsomal protein) would have an elevated AUC compared to other age groups. The sub-group of low metabolizing neonates exhibits higher AUCs and negligible metabolism rates (0 to 0.14 mg/hr), compared to high metabolizing neonates (CYP2E1 levels: 4.33 to 55.93 pmol/mg microsomal protein, metabolism rate: 0.23 to 1.43 mg/hr).

PBPK model simulations of inhalation exposure to 1 ppm toluene for 24-hr indicated that the end of exposure venous blood concentration would range from 0.015 to 0.050 μg/ml in neonates and from 0.008 to 0.023 μg/ml in all other groups of children. The simulated AUCs ranged from 0.30 to 1.01 μg/ ml×hr in neonates and from 0.16 to 0.43 μg/ml×hr in all other children (Figures 4-8C & 4-8D). Alike the observations of 17 ppm simulations, neonates under a month old showed greater variability compared to older children (Figure 4-8C vs 4-8D).

4.3.2. IVF-PK analysis

Figure 4-9 illustrates the population distributions of AUC of toluene, associated with 1 ppm exposure for 24 hr in adults and various sub-groups of children. The data for all sub-groups of children, except neonates, followed unimodal distribution and overlapped with the adult distribution. The 95th percentile AUCs for adolescent, child and infant (0.35 to 0.40 μg/ml×hr) are comparable to that of the adult group (0.38 μg/ml×hr) (Table 4-3). The neonates, on the other hand, exhibited higher AUC values corresponding to the 95th percentile (low metabolizing group: 0.99 μg/ml×hr, high metabolizing group: 0.63 μg/ml×hr). The intragroup variability factor was less than 1.5 and the adult-child factor was mostly within a factor of two (Table 4-3). The 95th percentile value of the low metabolizing neonates over the 50th percentile value in adults yielded an adult-child factor greater than 3.

4.3.3. Sensitivity analysis

The sensitivity of AUC to change in the numerical values of PBPK model parameters was conducted for the adults as well as for the various groups of children. Figure 4-10

shows the sensitivity ratio associated with each input parameter in each age group. This analysis indicates that liver:air partition coefficient, muscle blood flow and liver volume were among the least sensitive parameters for 24-hr $AUC_{toluene}$. In general, parameters that determine the pulmonary clearance (i.e., alveolar ventilation rate, blood:air partition coefficient) and metabolic clearance (liver blood flow rate, intrinsic clearance) were among the most sensitive parameters. Other parameters such as body weight, adipose tissue volume, muscle volume, cardiac output, adipose tissue blood flow, adipose tissue:air partition coefficient, rest of the body:air partition coefficient and muscle:air partition coefficient, exhibited medium sensitivity (i.e., sensitivity ratios ranging from 0.05 to 0.2). However, in low metabolizing neonates, unlike the other groups, the pulmonary clearance parameters (i.e., alveolar ventilation rate, blood:air partition coefficient) were clearly more sensitive than the metabolic clearance parameters (liver blood flow rate, intrinsic clearance) towards $AUC_{toluene}$.

4.4. DISCUSSION

The present study has illustrated a scientifically-sound way of evaluating the magnitude of the inter-individual and inter-age variability in internal dose based on available data on hepatic CYP2E1 levels and physiological growth in children. Subject-specific PBPK models were generated in this study to simulate the PK profiles of toluene in children of various age groups. Even though a number of studies have attempted to quantify the population distribution of internal dose and the IVF-PK, these studies have primarily been based on data or theoretical distributions for adults (Delic *et al.* 2000; Jonsson and Johanson 2001; Sweeney *et al.* 2001, 2004; Gentry *et al.* 2003). Only very few studies have focused on evaluating the effect on internal dose of age-specific changes in physiology and pharmacokinetic determinants (e.g., Price *et al.* 2003a; Clewell *et al.* 2004; Ginsberg *et al.* 2004). The default approach is to compute intrinsic clearance for children by scaling adult value of intrinsic clearance or adult maximal metabolic velocity on the basis of body surface ($BW^{0.75}$). Predictions based on body surface scaling do not reflect adequately the immaturity or developmental pattern of isozymes, and as such resulting internal dose calculations for children, particularly, neonates, may be

misleading. Figure 4-11 shows that the $AUC_{toluene}$ predicted on the basis of body surface-scaled metabolism rate (i.e., $BW^{0.75}$) does not correspond to those obtained on the basis of CYP2E1 levels in neonates. The present study represents the first attempt to use child-specific physiology and data on hepatic CYP2E1 content within a PBPK modeling framework to simulate the PK profiles and quantify inter-child variability in internal dose of an environmental contaminant (toluene).

The results of the present study indicate that AUC of toluene varied only by a factor of 3.9 even though liver CYP2E1 content varied by about a factor of 20 (Johnsrud *et al.* 2003). Due to the age-related changes in other physiological parameters, the PK variability is less than expected on the basis of age-related change in the levels of hepatic CYP2E1. The maximal factor, for AUC of toluene, was observed when the 95th percentile value of low metabolizing neonates was compared with the mean of the adult distribution. The estimated magnitude of IVF-PK for subgroups of infant, child, adolescent and adults was actually lower than the maximal adult-neonate factor of 3.9 or the default factor of 3. The magnitude of IVF-PK, in part, can be explained on the basis of CYP2E1 levels in neonates, children and adults.

There are several reports indicating that CYP2E1 is immature at birth followed by rapid onset and eventual maturation by 6 months to 1 year (Veiria *et al.* 1996, Cresteil 1998, Nakamura *et al.* 1998, Tanaka 1998). Using a more extensive analysis, Jonshrud *et al.* (2003) observed that maturation of hepatic CYP2E1 content occurred after 3 months, and expression comparable to adult levels was apparent after a year. Figure 4-12 indicates that the enzyme content is the more sensitive parameter than hepatic blood flow rate in these neonates whereas metabolism is more sensitive to blood flow in all other groups. In other words, hepatic metabolism of toluene would appear to be limited by enzyme content at birth and evolve gradually to a flow limited condition with increasing age. Not surprisingly, the rate of metabolism and AUC of toluene in high metabolizing neonates and older children are comparable to adults. However, in the neonatal group with CYP2E1 levels lower than 3.69 pmol/mg microsomal protein, the hepatic extraction ratio (E) is below 5%, explaining the higher AUC and lower metabolites formed in this group

compared to other age groups. Therefore, variability in the rate of metabolism and AUC in this group, as indicated by PBPK model simulations, is marked and a direct result of changes and variability in CYP2E1 levels. As the level of hepatic CYP2E1 increases (>1 month), toluene metabolism is limited by the liver blood flow rate (Figure 4-12) such that the inter-individual variation in enzyme content no longer has a direct influence on the inter-individual variability of the internal dose of toluene. The simulations obtained in this study are consistent with the fact that the inter-subject variability in CYP2E1 level is likely to impact toluene internal dose measures more significantly in neonates compared to older children and adults.

The present study also shows that the kinetics of toluene in older children is likely to be very similar to that reported in adults. This may be due to the fact that the pulmonary uptake of toluene and the respiratory clearance are both functions of body surface area. Further, mean hepatic CYP2E1 level has been reported to be 48.9 (11-130) pmol/mg microsomal protein in adults (Lipscomb *et al.* 2003), which is within the upper range of the pediatric CYP2E1 data set used in the current study (Johnsrud *et al.* 2003). Because of the similarity in the range of hepatic CYP2E1 content between older children and adults, the simulated pharmacokinetic profiles are similar in these groups. Since toluene is highly extracted in adults and older children (hepatic extraction ratio = 0.8), the inter-subject variability in AUC due to variability of hepatic CYP2E1 is minimal in these groups.

Hepatic clearance of toluene in this study was calculated using intrinsic clearance, representing the ratio of maximal velocity (Vmax) to Michaelis constant (Km) and is appropriate for use under first order conditions. The Km value, as part of the CL_{int} parameter, has limited influence on the AUC of toluene, particularly in low metabolizing neonates, since the enzyme content is too low to have any significant impact on the outcome. Further investigations are essential to characterize the age-related variability in liver microsomal protein levels as well as age-related change, if any, in the Michaelis constants to more confidently assess pharmacokinetic differences between children and adults.

The results of this modeling study suggest that the internal dose of toluene in children would differ by more than a factor of 3 from adults, if the hepatic CYP2E1 level is below 3.69 pmol/mg microsomal protein. At such low CYP2E1 levels, toluene metabolism is capacity-limited such that it would lead not only to increased AUC in neonates compared to adults but also lead to greater variability in internal dose reflective of the CYP2E1 content. The maximal factor representing adult-child difference estimated in this study (3.9) was obtained on the basis of the mean AUC in adults and the 95th percentile value in low metabolizing neonates. This factor relates only to pharmacokinetics and does not represent in any way the potential pharmacodynamic differences between adults and children which should also be accounted for. The conventional IVF-PK of 3.16 used in risk assessments for inhaled VOCs (Jarabek 1995) would then appear to be somewhat smaller than the adult-child factor calculated for toluene based on simulated parent chemical AUC. However, if the metabolite is the dose metric of relevance to toluene risk assessment, the default factor should be adequate since the neonates would appear to produce much lower levels of metabolites than adults because of the low levels of hepatic CYP2E1.

In conclusion, the subject-specific PBPK model developed in this study facilitates the evaluation of the quantitative relationship among hepatic CYP2E1 levels, age-specific physiology, and internal dose metrics of toluene. The CYP2E1 content-based PK model developed in the present study can be applied to evaluate the inter-child and population variability of other VOCs exhibiting a range of hepatic extraction ratios and blood solubility characteristics. The IVF-PK calculated for such CYP2E1 substrates for various exposures (inhalation, dermal and oral) and scenarios (repeated exposures, multichemical exposures and induction of metabolism) would contribute to the refinement or justification of the default interindividual variability factors used in specific risk assessments.

4.5. REFERENCES

Agency for Toxic Substances and Disease Registry (ATSDR). (2000). Toxicological profile for toluene. U.S. Department Of Health And Human Services, Atlanta, Georgia.

American Conference of Governmental Industrial Hygienists (ACGIH). (2005). The documentation of the Threshold Limit Values and Biological Exposure Indices. ACGIH, Cincinnati, Ohio.

Benignus VA., Bushnell PJ., and Boyes, W.K. (1998). A dosimetric analysis of behavioral effects of acute toluene exposure in rats and humans. Toxicol Sci. 43: 186-95.

Benignus VA., Bushnell PJ., and Boyes, W.K. (2005). Toward cost-benefit analysis of acute behavioral effects of toluene in humans. Risk Anal. 25: 447-56.

Cahalan M.K., Johnson B.H., and Edger E.I. (1981). Relationship of concentrations of halothane and enflurane to their metabolism and elimination in man. Anesthesiology. 54, 3-8.

Clewell H.J., and Andersen M.E. (1996). Use of physiologically based pharmacokinetic modeling to investigate individual versus population risk. Toxicology. 111, 315-29.

Clewell, H.J., Gentry, P.R., Covington, T.R., Sarangapani, R., and Teeguarden, J.G. (2004). Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. Toxicol Sci. 79,381-93.

Clewell H.J,. Teeguarden J., McDonald T., Sarangapani R., Lawrence G., Covington T., Gentry R., and Shipp A. (2002). Review and evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. Crit Rev Toxicol. 32, 329-89.

Cresteil, T. (1998). Onset of xenobiotic metabolism in children: Toxicological implications. Food Addit. Contam. 15(Suppl.), 45–51.

Delic J.I., Lilly P.D., MacDonald A.J., and Loizou G.D. (2000). The utility of PBPK in the safety assessment of chloroform and carbon tetrachloride. Regul Toxicol Pharmacol. 32, 144-55.

Dorne J.L. and Renwick A.G. (2005). The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. Toxicol. Sci. 86. 20-26.

Dorne J.L., Walton K., and Renwick A.G. (2005). Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. Food Chem Toxicol. 43. 203-16.

Dourson M., Charnley G., and Scheuplein R. (2002). Differential sensitivity of children and adults to chemical toxicity. II. Risk and regulation. Regul Toxicol Pharmacol. 35, 448-67.

Foo, S.C., Jeyaratnam, J., D. Koh, D. (1990). Chronic neurobehavioral effects of toluene. Br J Ind Med. 47,480-484.

Gentry, P.R., Covington, T.R., and Clewell, H.J. (2003). Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. Regul Toxicol Pharmacol. 38, 1-16.

Guengerich F.P., Kim D.H., and Iwasaki M. (1991). Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. Chem Res Toxicol. 4, 168-79.

Ginsberg G, Hattis D, Russ A., and Sonawane B. (2004). Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: implications for assessing children's risks from environmental agents. J Toxicol Environ Health 27: 297-329.

Haddad S., Tardif, R., Viau, C., and Krishnan K. (1999). A modeling approach to account for pharmacokinetic interactions in the calculation of biological hazard index for chemical mixtures. Toxicol Lett 108: 303-308.

Haddad S., Pelekis M., and Krishnan K. (1996). A methodology for solving physiologically based pharmacokinetic models without the use of simulation softwares. Toxicol Lett. 85, 113-26.

Haddad S., Restieri C., and Krishnan K. (2001). Characterization of age-related changes in body weight and organ weights from birth to adolescence in humans. J Toxicol Environ Health A. 64, :453-64.

International Program on Chemical Safety (IPCS). (2001). Guidance Document for the Use of Data in Development of Chemical-Specific Adjustment Factors (CSAFs) for Interspecies Differences and Human Variability in Dose/Concentration–Response Assessment. World Health Organization, Geneva.

Jarabek A.M. (1995). The application of dosimetry models to identify key processes and parameters for default dose-response assessment approaches. Toxicol Lett. 79, 171-84.

Johnsrud E.K., Koukouritaki S.B., Divakaran K., Brunengraber L.L., and Hines R.N., and McCarver D.G. (2003). Human hepatic CYP2E1 expression during development. J Pharmacol Exp Ther. 307, 402-7.

Jonsson F., and Johanson G. (2001). Bayesian estimation of variability in adipose tissue blood flow in man by physiologically based pharmacokinetic modeling of inhalation exposure to toluene. Toxicology. 157, 177-93.

Kao S.C., Bell E.F., Brown B.P., and Smith W.L. (1996). Duplex Doppler sonography of changes in portal vein flow in healthy term newborn infants after feeding. J Ultrasound Med. 15, 121-5.

Kedderis G.L., and Lipscomb J.C. (2001). Application of in vitro biotransformation data and pharmacokinetic modeling to risk assessment. Toxicol Ind Health. 17, 315-21.

Koukouritaki S.B., Manro J.R., Marsh S.A., Stevens J.C., Rettie A.E., McCarver D.G., and Hines R.N. (2004). Developmental expression of human hepatic CYP2C9 and CYP2C19. J Pharmacol Exp Ther. 308, 965-74.

Lipscomb J.C., Barton H.A., Tornero-Velez R., Evans M.V., Alcasey S., Snawder J.E., and Laskey J. (2004). The metabolic rate constants and specific activity of human and rat hepatic cytochrome P-450 2E1 toward toluene and chloroform. J Toxicol Environ Health A. 67, 537-53.

Lipscomb J.C., Teuschler L.K., Swartout J., Popken D., Cox T., and Kedderis G.L. (2003). The impact of cytochrome P450 2E1-dependent metabolic variance on a risk-relevant pharmacokinetic outcome in humans. Risk Anal. 23, 1221-38.

Lof, A., Wigaeus Hjelm, E., Colmsjo, A., Lundmark, B.O., Norstrom, A., and Sato A. (1993). Toxicokinetics of toluene and urinary excretion of hippuric acid after human exposure to 2H8-toluene. Br J Ind Med. 50, 55-9.

Nakajima T., Elovaara E., Park S.S., Gelboin H.V., and Vainio H. (1991). Immunochemical detection of cytochrome P450 isozymes induced in rat liver by n-hexane, 2-hexanone and acetonyl acetone. Arch Toxicol. 65, 542-7.

Nakajima T, Wang RS. (1994). Induction of cytochrome P450 by toluene. Int J Biochem. 26, 1333-40.

Nakajima T, Wang RS, Elovaara E, Gonzalez FJ, Gelboin HV, Raunio H, Pelkonen O, Vainio H, Aoyama T. (1997). Toluene metabolism by cDNA-expressed human hepatic cytochrome P450. Biochem Pharmacol. 53,271-7.

Nakamura, H., Tanaka, E., Ishikawa, A., Fukao, K., Tsuji, K., and Ohkawa, H. (1998). Age-related changes in hepatic drug-oxidizing activity using trimethadione as a probe drug in humans. Hepatol. Res. 12, 85–92.

National Toxicology Program (NTP). (1990) Toxicology and carcinogenesis studies of toluene (CAS No. 108-88-3) in F344/N rats and B5C3F1 mice (inhalation studies). Public Health Service, U.S. Department of Health and Human Services; NTP TR 371.

Ogata, M. (1984). Estimation of solvent concentrations in ambient air from urinary metabolite levels of workers exposed to solvents. Ind Health. 22, 319-24.

Pierce C.H., Dills R.L., Silvey G.W., and Kalman D.A. (1996). Partition coefficients between human blood or adipose tissue and air for aromatic solvents. Scand J Work Environ Health. 22, 112-8.

Poulin, P. and Krishnan, K. (1999). Molecular structure-based prediction of the toxicokinetics of inhaled vapors in humans. Int J Toxicol. 18, 7-18.

Price K., Haddad S., and Krishnan K. (2003a). Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. J Toxicol Environ Health A. 66, 417-33.

Price P.S., Conolly R.B., Chaisson C.F., Gross E.A., Young J.S., Mathis E.T., and Tedder D.R. (2003b). Modeling interindividual variation in physiological factors used in PBPK models of humans. Crit Rev Toxicol. 33, 469-503.

Rumchev K, Spickett J, Bulsara M, Phillips M and Stick S. (2004) Association of domestic exposure to volatile organic compounds with asthma in young children. Thorax 59, 746-751.

Sexton K, Adgate JL, Church TR, Ashley DL, Needham LL, Ramachandran G, Frederickson AL and Ryan AD. (2005). Children,s exposure to volatile organic compounds as determined by longitudinal measurements in blood. Environ Health Perspect 113, 342-349.

Sonnier, M., and Cresteil, T. (1998). Delayed ontogenesis of CYP1A2 in the human liver. Eur J Biochem. 251, 893-8.

Stevens J.C., Hines R.N., Gu C., Koukouritaki S.B., Manro J.R., Tandler P.J., and Zaya M.J. (2003). Developmental expression of the major human hepatic CYP3A enzymes. J Pharmacol Exp Ther. 307, 573-82.

Sweeney L.M., Kirman C.R., Morgott D.A., and Gargas M.L. (2004). Estimation of interindividual variation in oxidative metabolism of dichloromethane in human volunteers. Toxicol Lett. 154, 201-16.

Sweeney L.M., Tyler T.R., Kirman C.R., Corley R.A,. Reitz R.H., Paustenbach D.J., Holson J.F., Whorton M.D., Thompson K.M., and Gargas M.L. (2001). Proposed occupational exposure limits for select ethylene glycol ethers using PBPK models and Monte Carlo simulations. Toxicol Sci. 62, 124-39.

Tanaka, E. (1998). Clinically important pharmacokinetic drug-drug interactions: Role of cytochrome P450 enzymes. J. Clin. Pharm. Ther. 23, 403–416.

Tardif R., Charest-Tardif G., Brodeur J., and Krishnan K. (1997). Physiologically based pharmacokinetic modeling of a ternary mixture of alkyl benzenes in rats and humans. Toxicol Appl Pharmacol 144, 120-134.

Tardif R., Lapare S., Charest-Tardif G., Brodeur J., and Krishnan K. (1995). Physiologically-based pharmacokinetic modeling of a mixture of toluene and xylene in humans. Risk Anal. 15, 335-42.

Tardif R., Truchon G., and Brodeur J. (1998). Comparison of hippuric acid and o-cresol in urine and unchanged toluene in alveolar air for the biological monitoring of exposure to toluene in human volunteers. Appl Occup Environ Hyg. 13, 127-132.

Tassaneeyakul W., Birkett D.J., Edwards J.W., Veronese M.E., Tassaneeyakul W., Tukey R.H., and Miners J.O. (1996). Human cytochrome P450 isoform specificity in the regioselective metabolism of toluene and o-, m- and p-xylene. J Pharmacol Exp Ther. 276, 101-8.

Thomas R.S., Bigelow P.L., Keefe T.J., and Yang R.S. (1996). Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. Am Ind Hyg Assoc J. 57, 23-32.

US Environmental Protection Agency (US EPA). (2001). Risk Assessment Guidance for Superfund Volume 3 Part A: Process for Conducting Probabilistic Risk Assessment (RAGS 3A). Office of Research and Development. USEPA, Washington, DC. EPA/540/R-02/002.

US Environmental Protection Agency (US EPA). (2005). Toxicological review of toluene in support of summary information on the Integrated Risk Information System (IRIS). USEPA, Washington, DC. EPA/635/R-05/004.

Vieira, I., Sonnier, M., and Cresteil, T. (1996). Developmental expression of CYP2E1 in the human liver. Hypermethylation control of gene expression during the neonatal period. Eur. J. Biochem. 238, 476–483.

White, D.R., Widdowson, E.M., and Woodard, H.Q. (1991). The composition of body tissues. (II) Fetus to young adult. Br J Radiol 64, 149–159.

Wilkinson, G.R., and Shand, D.G. (1975). Commentary: a physiological approach to hepatic drug clearance. Clin Pharmacol Ther. 18, 377-90.

Table 4-1 Mean (range) of physiological parameters used for PBPK modeling[a].

Parameters	Neonate (< 1 mo)	Infant (1 mo - 1 yr)	Child (1 - 11 yr)	Adolescent (12 - 17 yr)	Adult[b] (18+ yr)
Sample size	28	48	27	13	
Body weight (kg)	1.8 (0.3 - 4.5)	5.7 (2.4 - 9.1)	20.9 (7.3 - 56.7)	62.1 (37.6 - 97.5)	70.0 (47-103)
Tissue volumes (L)					
Liver	0.07 (0.004 - 0.20)	0.23 (0.09 - 0.39)	0.61 (0.25 - 1.36)	1.42 (0.91 - 1.88)	1.82 (0.73 – 2.91)
Fat	0.36 (0.06 - 0.78)	0.84 (0.50 - 1.19)	3.57 (0.95 - 10.77)	11.43 (6.53 - 18.53)	13.30 (1.33 - 25.27)
Muscles and skin	0.35 (0.05 - 1.29)	2.02 (0.43 - 4.04)	11.38 (2.94 - 35.15)	37.85 (21.34 -60.46)	43.40 (4.59 - 82.46)
Rest of the body	0.74 (0.11 - 1.67)	1.84 (0.95 - 2.40)	2.88 (1.95 - 4.33)	5.12 (3.72 - 7.39)	11.48 (4.34 - 18.37)
Cardiac output (L/min)	0.32 (0.08 - 0.85)	1.15 (0.41 - 1.96)	3.64 (1.54 - 5.95)	6.67 (5.76 - 8.89)	6.96 (2.76 - 16.08)
Alveolar ventilation(L/min)	0.45 (0.12 - 0.85)	0.94 (0.61 - 1.20)	2.37 (1.05 - 5.95)	6.24 (4.09 - 8.89)	6.96 (2.76 - 16.08)
Tissue blood flows (L/min)					
Liver	0.09 (0.01 - 0.23)	0.18 (0.11 - 0.37)	0.48 (0.18 - 1.35)	1.37 (0.77 - 1.87)	1.81 (0.02 – 3.60)
Fat	0.03 (0.004 - 0.09)	0.09 (0.03 - 0.13)	0.29 (0.11 -0.59)	0.44 (0.29 - 0.70)	0.35 (0.19 – 0.50)
Muscles and skin	0.01 (0.002 - 0.03)	0.07 (0.02 - 0.14)	0.45 (0.10 - 1.49)	1.51 (0.85 - 2.22)	1.74 (0.31 – 5.82)
Rest of the body	0.20 (0.07 - 0.59)	0.81 (0.10 - 1.47)	2.42 (1.02 -3.54)	3.35 (2.73 - 4.54)	3.06 (0.17 – 3.31)
Metabolism					
CYP2E1 liver content (pmol/mg MSP)	9.1 (0.0 - 55.9)	40.2 (14.3 - 86.1)	43.5 (18.2 -94.7)	62.2 (23.1 - 86.1)	48.9[c] (11-130)
Intrinsic clearance (L/min)	0.03 (0.00 - 0.17)	0.37 (0.10 - 1.01)	1.05 (0.21 – 5.09)	3.49 (1.37 – 5.93)	3.52 (0.32 – 14.96)

[a] CYP2E1 liver content, body weights and liver volumes are from Johnsrud *et al.* (2003), whereas all other parameters were calculated on the basis of the body weight of children (Haddad *et al.* 2001, Price *et al.* 2003a).

[b] Adult mean values were from Tardif et al .(1995) and distribution and range were calculated based on Thomas et al. (1996).

[c] Adult CYP2E1 liver content values were based on Lipscomb et al. (2004).

Table 4-2 Partition coefficients for human PBPK modeling of toluene[a].

Partition coefficients	Value
Blood : air	15.6
Liver : blood	5.36
Fat : blood	65.45
Muscles and skin : blood	1.78
Rest of the body: blood	5.36

[a]The tissue:blood partition coefficients were calculated as rat tissue:air divided by human blood:air. The rest of the body compartment was assumed to have the same partition coefficient as the liver. Data from Tardif *et al.* (1995).

Table 4-3 **Intragroup and adult-child factor derived from area under the venous blood concentration vs time curves (AUC) of toluene in children and adults[1].**

Age group	Percentile values of AUC (μg/ml×hr)			Intragroup Factor[2]	Adult-Child Factor[3]
	5%	50%	95%		
Adult	0.17	0.26	0.38	1.48	
Adolescent (12-17 yr)	0.22	0.25	0.35	1.38	1.35
Child (1-11 yr)	0.22	0.28	0.38	1.36	1.49
Infant (1 mo - 1 yr)	0.24	0.31	0.40	1.29	1.57
Neonate (< 1 month)					
low metabolizers	0.77	0.93	0.99	1.07	3.88
high metabolizers	0.31	0.48	0.63	1.31	2.46

[1]AUCs were calculated for a 24-hour period (exposure duration: 24 hr, exposure concentration: 1 ppm);
[2]The intragroup variability factor was calculated as the ratio of 95th percentile value over the 50th value for the same age group;
[3]The adult-child variability factor was calculated as the ratio of the 95th percentile value for child over the 50th percentile value for the adult.

FIGURE LEGENDS

Figure 4-1 Subject-specific data on body weight (A), liver weight (B), and hepatic CYP2E1 protein levels (C, D) in children. Data from Johnsrud *et al.* (2003).

Figure 4-2 Schematic representation of the PBPK model for toluene.

Figure 4-3 Quantitative relationships between average body weight (BW) and tissue volumes ((A) fat, (B) muscle and skin and (C) skeleton). Data from Haddad *et al.* (2001). The solid lines were generated using the following regression equations:
$V_{fat} = 4E\text{-}06xBW^4 - 0.0005xBW^3 + 0.0207xBW^2 - 0.1127xBW + 0.8619$
$V_{skeleton} = 4E\text{-}07xBW4 - 5E\text{-}05xBW^3 + 0.001xBW^2 + 0.1177xBW + 0.1311$
$V_{muscle} = 0.6056xBW - 1.4558$

Figure 4-4 Intrinsic clearance of toluene calculated on the basis of CYP2E1 content and liver weight of each child [(A) neonates (< 1 month) and (B) older children]. Subject-specific data on CYP2E1 content and liver weight were obtained from Johnsrud *et al.* (2003).

Figure 4-5 Inhalation PBPK model simulations of venous blood concentrations in children (n=116; from birth to 17 years old) exposed for 7-hr to 17 ppm of toluene. This exposure concentration and duration correspond to those of a previous study in which adult volunteers were exposed to toluene for collection of data on blood concentrations (represented as symbols) (Tardif *et al.* 1997).

Figure 4-6 Maximal venous blood concentration (Cmax) of toluene in (A) neonates (Δ: high metabolizers, ▲: low metabolizers) and (B) older children (○). The simulations were based on a 7-hr exposure to 17 ppm of toluene (Tardif *et al.* 1997).

Figure 4-7 The association between CYP2E1 concentration and hepatic clearance in children of various age groups (A), along with the hepatic extraction ratio in various age groups of children (B) (neonate I: low metabolizers, II: high metabolizers).

Figure 4-8 Simulated area under the venous blood concentration vs time curve (AUC) over a 24-hr period in neonates (A and C) and older children (B and D) exposed to 17 ppm for 7 hr (A and B; : high metabolizers, ▲: low metabolizers) or to 1 ppm for 24 hr (C and D; □: high metabolizers, ■: low metabolizers).

Figure 4-9 Cumulative probability density distribution of the area under the venous blood concentration vs time curve over a 24-hr period (AUC) for toluene

in adults (dotted lines) and children (solid lines; (A) low metabolizing neonates (< 1 month), (B) high metabolizing neonate (< 1 month), (C) infant (1 month to 1 year old), (D) child (1 to 11 years old), and (E) adolescent (12 to 17 years old)).

Figure 4-10 The sensitivity of toluene area under the blood concentration vs time (AUC, μg/ml×hr) to PBPK model parameters in various age groups (from left to right: ⊟ low metabolizing neonates, □ high metabolizing neonates, ▫ infants, ⊡ child, ■ adolescent and ▩ adults). The sensitivity ratios were calculated as the change in AUC for a 5% change in the value of input parameters (body weight BW, intrinsic clearance CL_{int}, partition coefficients (blood:air Pb, fat:air Pf, liver:air Pl, rest of the body:air Pr, muscle:air Ps), cardiac output Qc, tissue blood flows Qx and tissue volumes Vx (adipose tissues f, hepatic l, muscle s)). AUCs were obtained for 24-hr exposure to 1 ppm toluene.

Figure 4-11 Comparison of area under the venous blood concentration vs time (AUC) obtained by using CYP2E1 content-based metabolism rate in children with that obtained on the basis of metabolism rate scaled to body weight ($BW^{0.75}$) of children. Solid line represents perfect correlation between the two outcomes. PBPK model simulations are presented as symbols (■: neonates (< 1 month), ♦: infant (1 month to 1 year old), Δ: child (1 to 11 years old), ○: adolescent (12 to 17 years old), and ●: adult (18 and over)) and were obtained for 24-hr inhalation exposure to 1 ppm toluene.

Figure 4-12 The sensitivity of hepatic clearance (CL_h, L/min) to input parameters (hepatic blood flow Ql, and intrinsic clearance CL_{int}) in various age groups (from left to right: ⊟ low metabolizing neonates, □ high metabolizing neonates, ▫ infants, ⊡ child, ■ adolescent and ▩ adults). The sensitivity ratios were calculated as the change in Cl_h for a 5% change in the value of the input parameters.

Figure 4-1

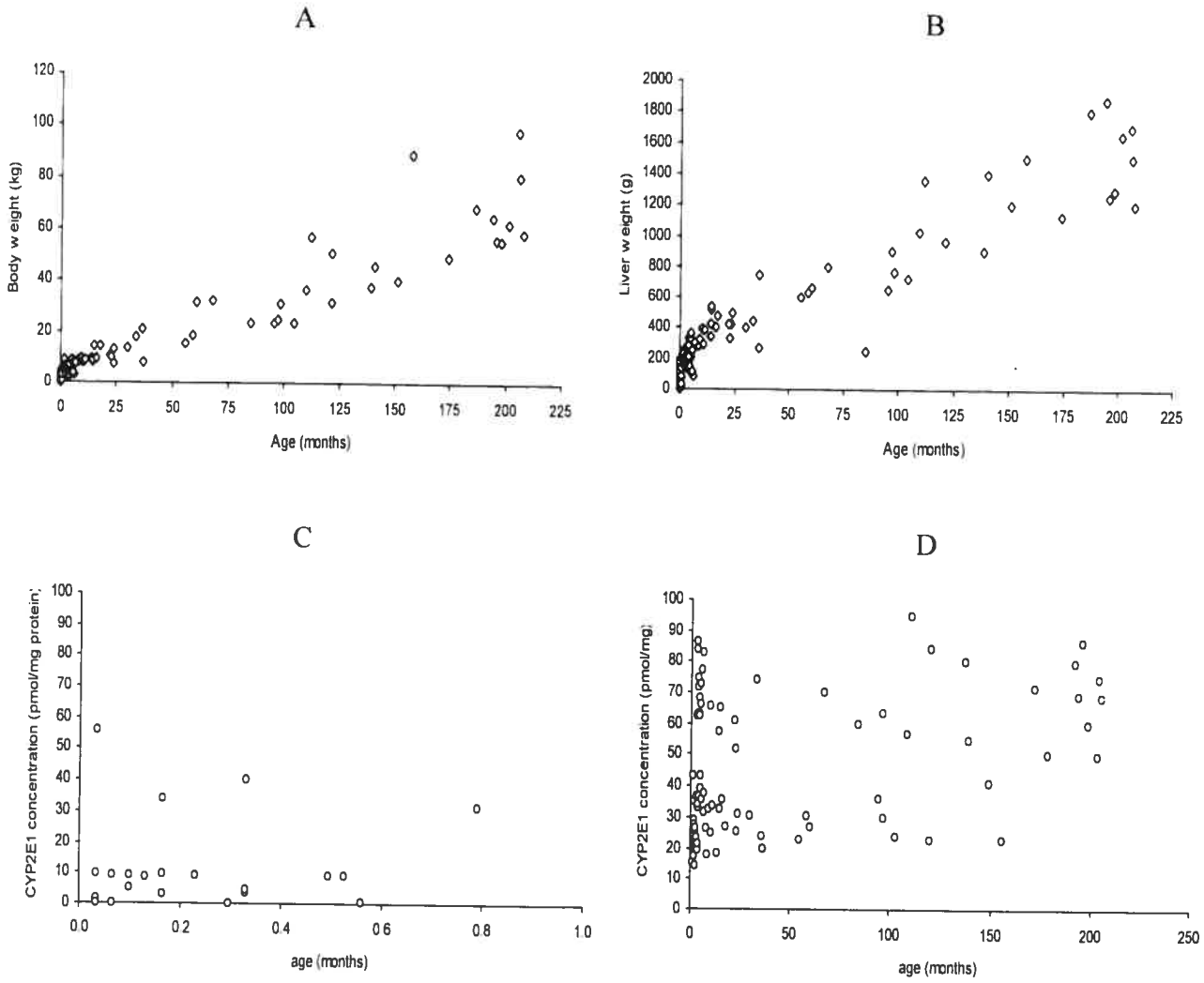

A
Body weight (kg)
Age (months)
B
Liver weight (g)
Age (months)
C
CYP2E1 concentration (pmol/mg protein)
age (months)
D
CYP2E1 concentration (pmol/mg)
age (months)

Figure 4-2

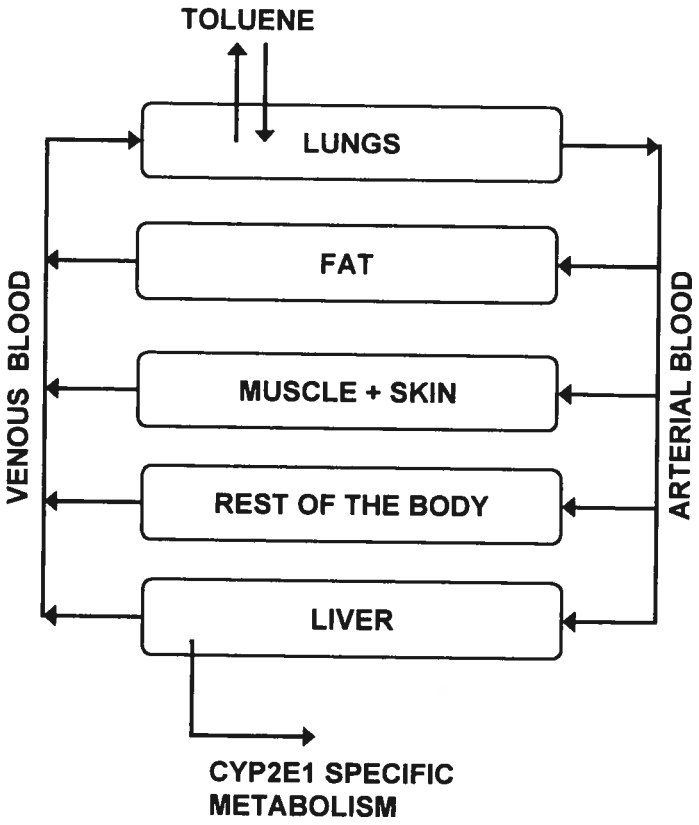
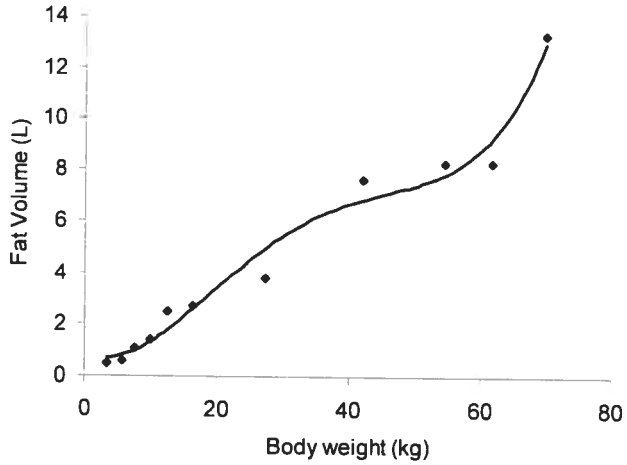
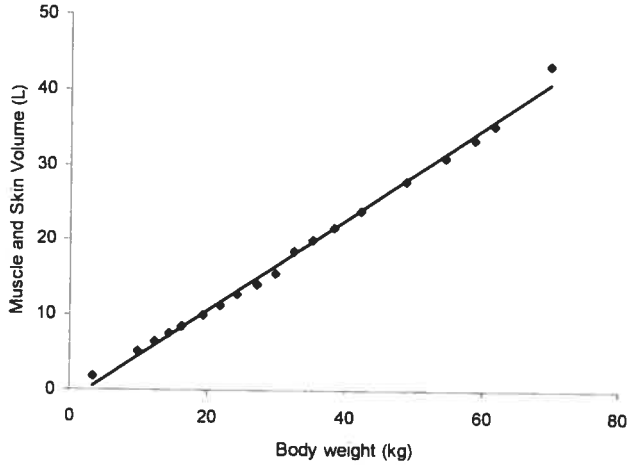

TOLUENE
LUNGS
FAT
MUSCLE + SKIN
REST OF THE BODY
LIVER
VENOUS BLOOD
ARTERIAL BLOOD
CYP2E1 SPECIFIC
METABOLISM

Figure 4-3

A



B



C

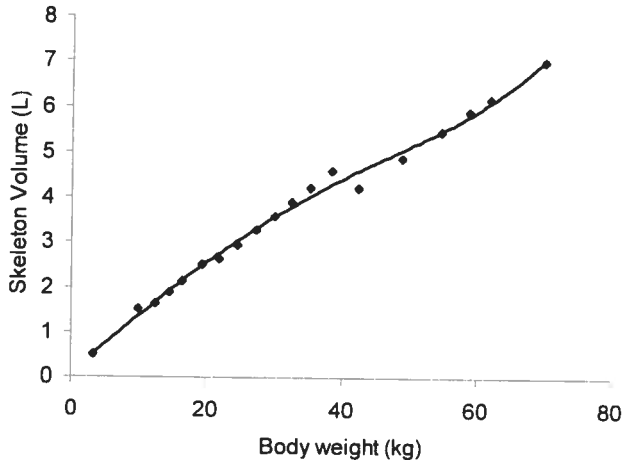


Figure 4-4

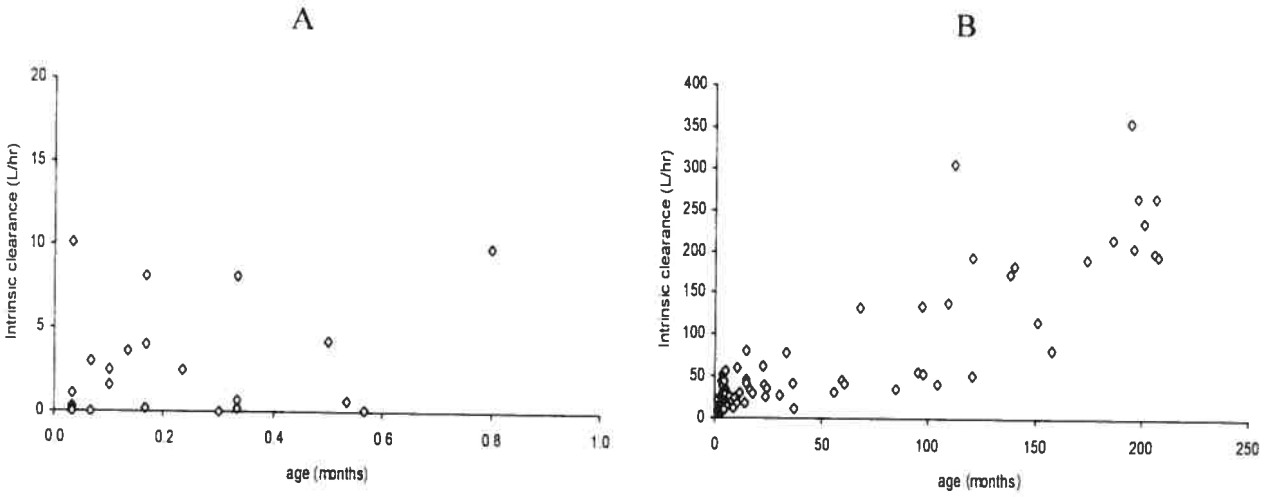

A
B
Intrinsic clearance (L/hr)
age (months)
0
5
10
15
20
0.0
0.2
0.4
0.6
0.8
1.0
50
100
150
200
250
300
350
400

Figure 4-5

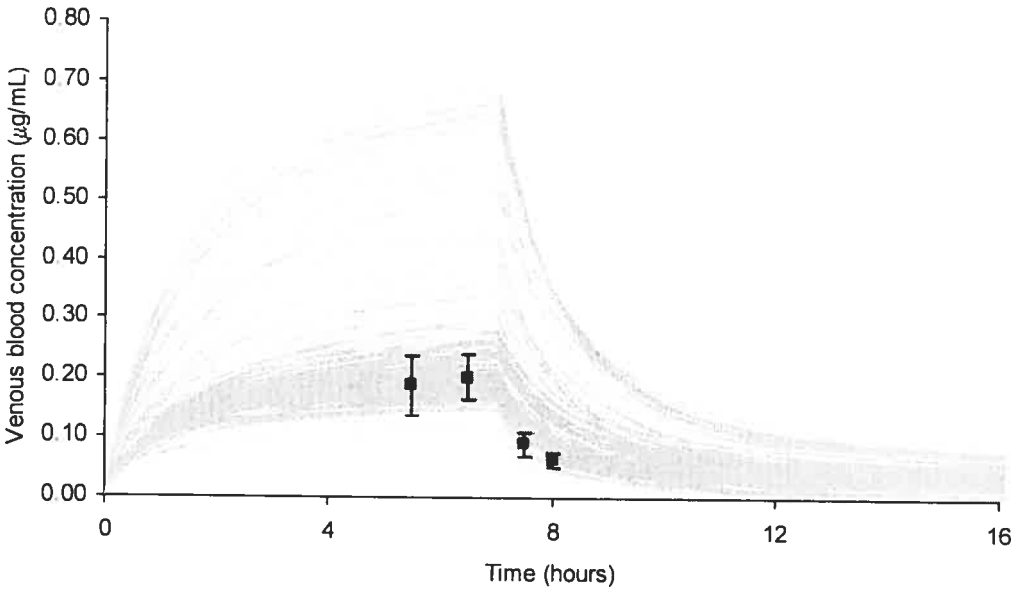

0.80
0.70
0.60
0.50
0.40
0.30
0.20
0.10
0.00
Venous blood concentration (µg/mL)
0
4
8
12
16
Time (hours)

Figure 4-6

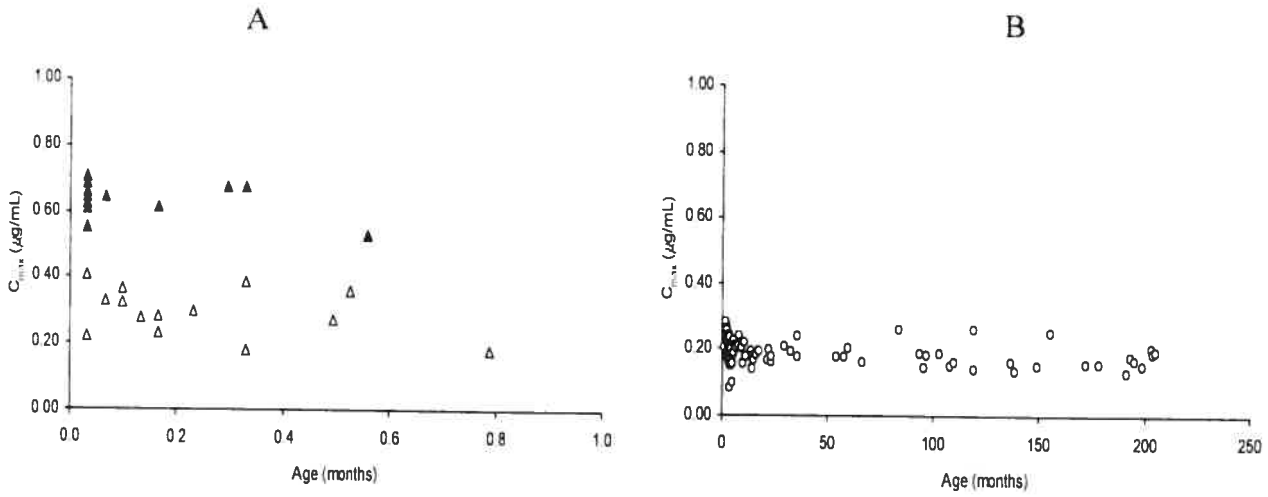
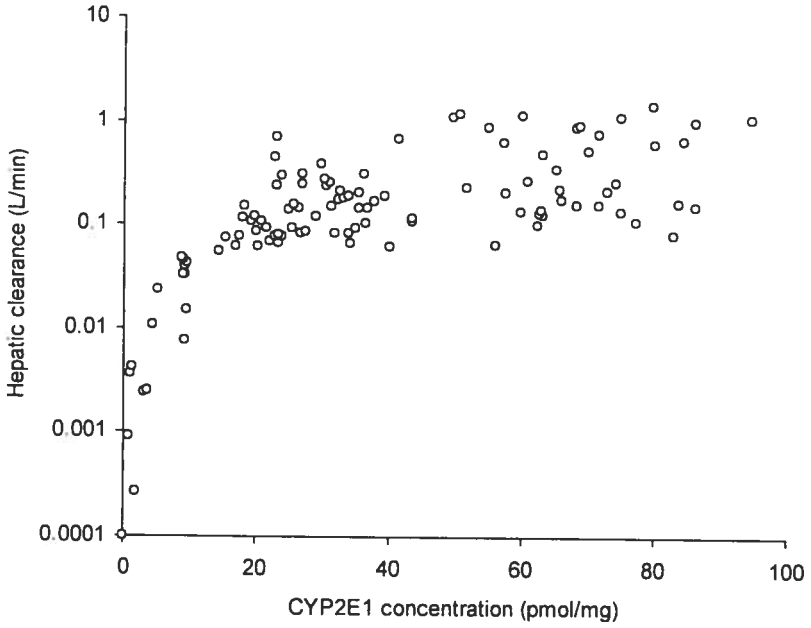

A
B
1.00
0.80
0.60
0.40
0.20
0.00
Age (months)
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250

Figure 4-7

A


10
1
0.1
0.01
0.001
0.0001
Hepatic clearance (L/min)
0
20
40
60
80
100
CYP2E1 concentration (pmol/mg)

B

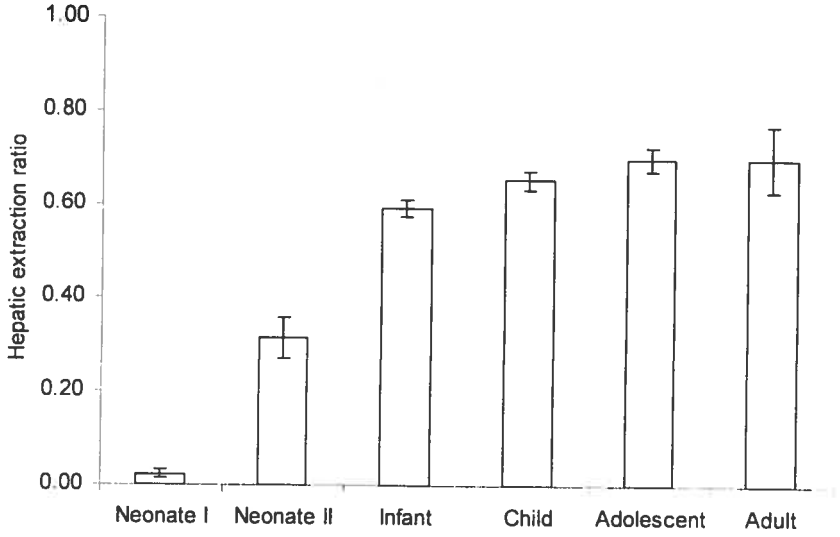

1.00
0.80
0.60
0.40
0.20
0.00
Hepatic extraction ratio
Neonate I
Neonate II
Infant
Child
Adolescent
Adult

Figure 4-8

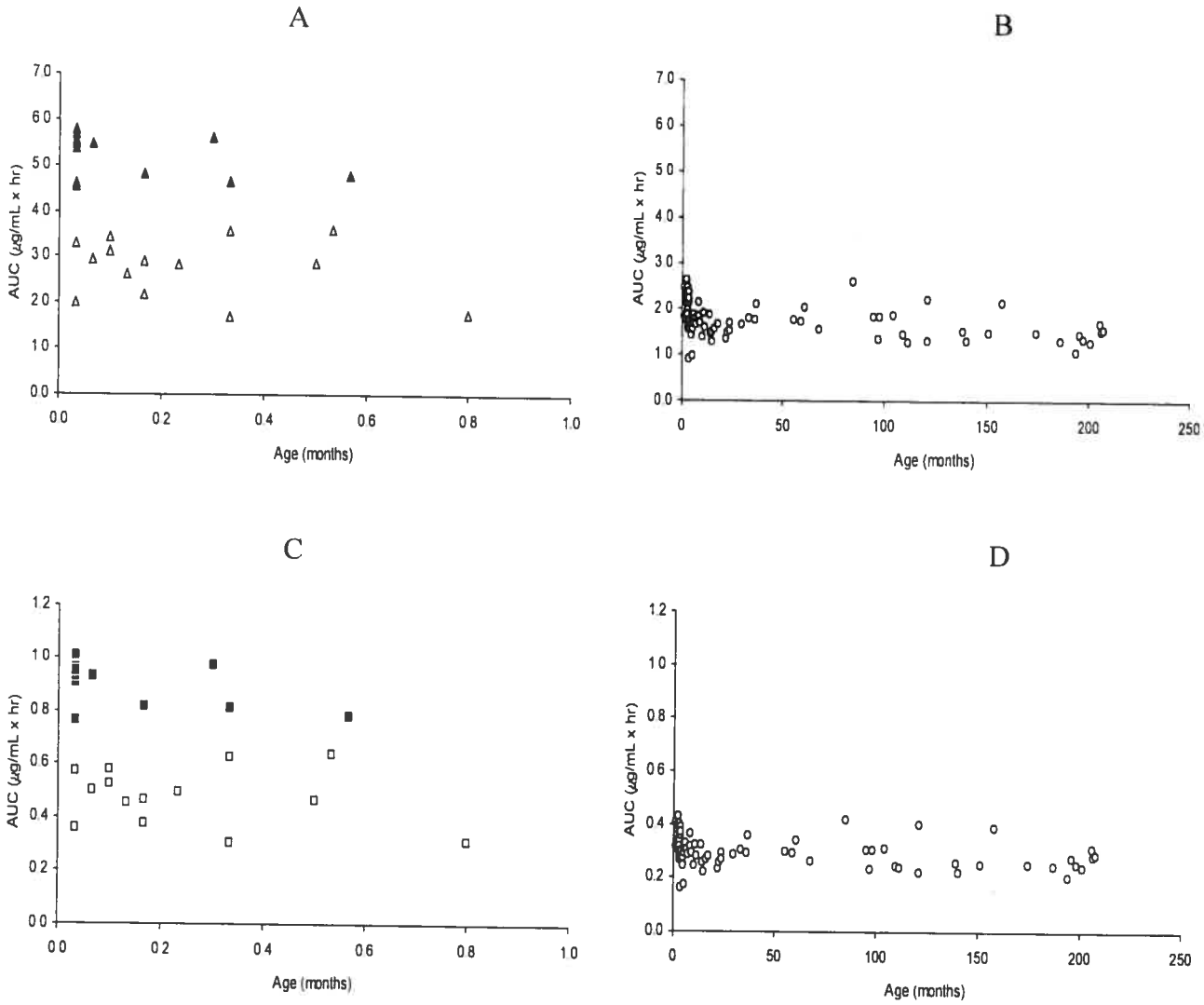

A
B
C
D
AUC (µg/mL x hr)
Age (months)
0.0
0.2
0.4
0.6
0.8
1.0
0
50
100
150
200
250

Figure 4-9

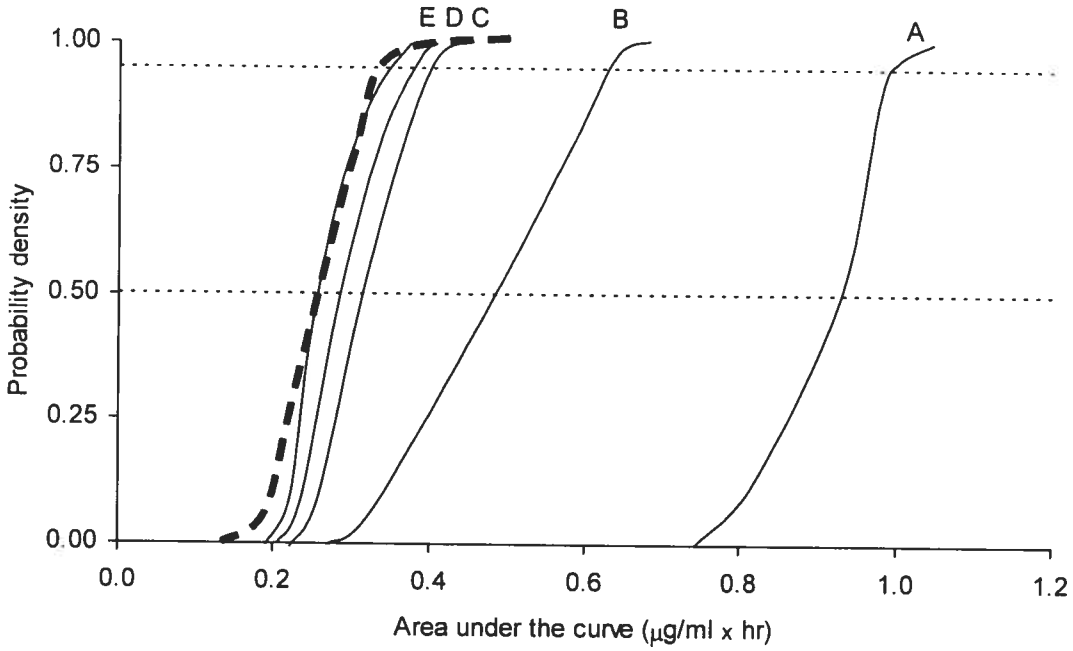

E D C
B
A
1.00
0.75
0.50
0.25
0.00
Probability density
0.0
0.2
0.4
0.6
0.8
1.0
1.2
Area under the curve (μg/ml x hr)

Figure 4-10

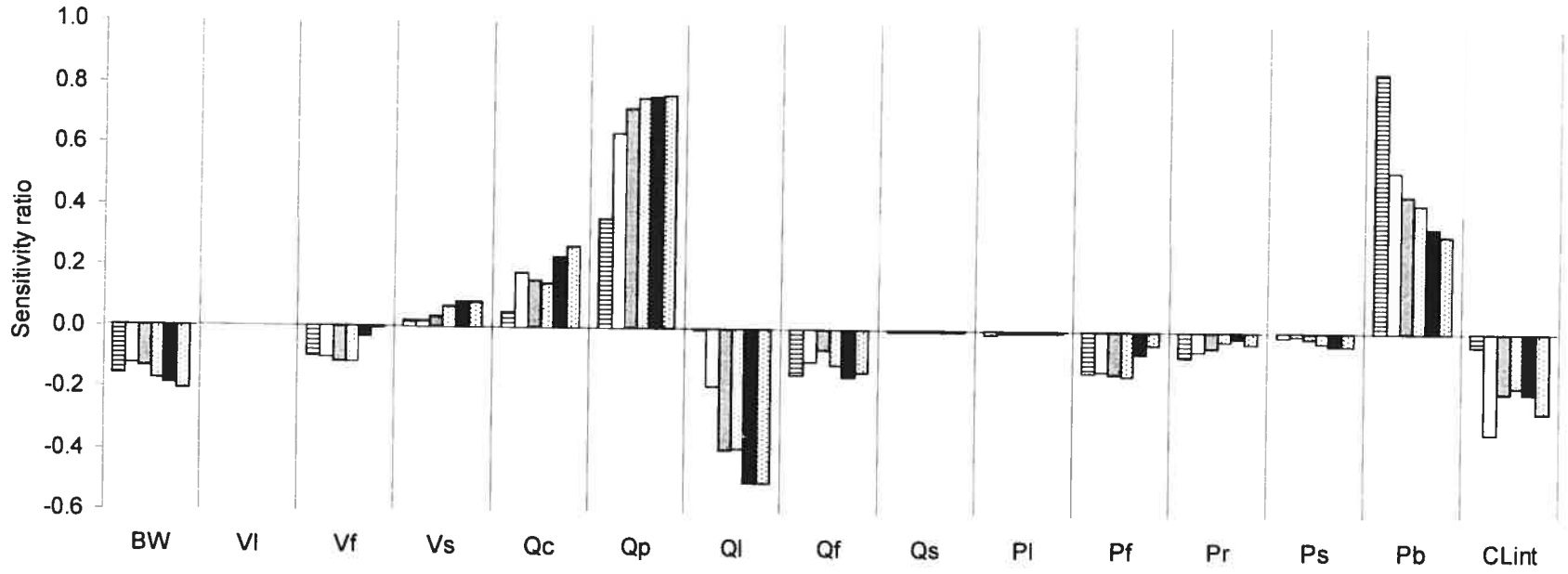

1.0
0.8
0.6
0.4
0.2
0.0
-0.2
-0.4
-0.6
Sensitivity ratio
BW
Vl
Vf
Vs
Qc
Qp
Ql
Qf
Qs
Pl
Pf
Pr
Ps
Pb
CLint

Figure 4-11

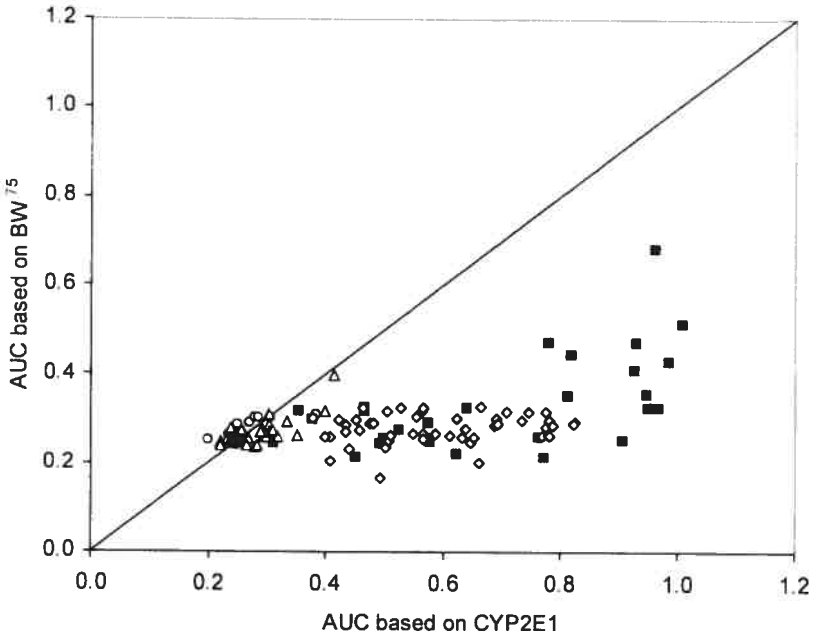
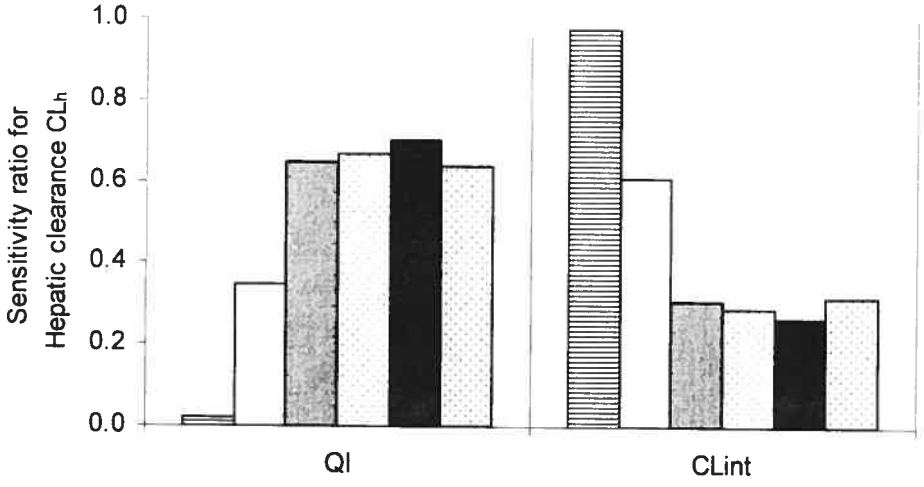

1.2
1.0
0.8
0.6
0.4
0.2
0.0
AUC based on BW^.75
0.0
0.2
0.4
0.6
0.8
1.0
1.2
AUC based on CYP2E1

Figure 4-12


Sensitivity ratio for
Hepatic clearance CL_h
1.0
0.8
0.6
0.4
0.2
0.0
Ql
CLint

CHAPITRE V

5. ARTICLE III

Bayesian analysis of the inhalation pharmacokinetics of methyl tert-butyl ether and its metabolite tert-butanol in humans.

Nong, A., Ernstgard, L., Krishnan, K., and Johanson, G.

Nong, A., Ernstgard, L., Krishnan, K., and Johanson, G. 2007. Bayesian analysis of the inhalation pharmacokinetics of methyl tert-butyl ether and its metabolite tert-butanol in humans. Journal of Toxicology and Environmental Health A. xx, xxxx-xxxx. (soumis)

Bayesian analysis of the inhalation pharmacokinetics of methyl *tert*-butyl ether and its metabolite *tert*-butanol in humans

Andy Nong[1], Lena Ernstgard[2], Kannan Krishnan[1] and Gunnar Johanson[2]

[1] TOXHUM, Université de Montréal, Montreal, Canada.

[2] Work Environment Toxicology, Karolinska Institutet, Stockholm, Sweden.

Correspondence to:

K. Krishnan, DSEST, University of Montreal, 2375 Cote Ste Catherine Office 4105, Montreal, PQ, Canada, H3T 1A8. Email: Kannan.Krishnan@umontreal.ca, Fax: 514-343-2200.

ABSTRACT

The objective of the present study was to conduct Bayesian analysis of the inhalation pharmacokinetics of methyl *tert*-butyl ether (MTBE) in humans. The approach consisted of the use of Markov Chain Monte Carlo (MCMC) simulation in a physiologically-based pharmacokinetic (PBPK) model, for computing posterior distributions of PBPK model parameters based on prior information on physiological and physicochemical data, as well as experimental data on the inhalation pharmacokinetics of MTBE and its metabolite *tert*-butanol (TBA) in humans. Previously published data on blood concentrations of MTBE and TBA in individuals (n=10) exposed to 5 - 50 ppm of MTBE, while performing light workload (50 W), were retrieved. The distribution of blood concentrations of MTBE and TBA, obtained with the resulting model, was comparable with the individual-specific experimental values. The convergence of the posterior estimates on the sensitive parameters (working cardiac output, working alveolar ventilation, MTBE blood:air partition coefficient, intrinsic clearance of MTBE and TBA, and TBA volume of distribution) was obtained after 30,000 iterations. Monte Carlo simulations for a general healthy adult population were then conducted using the PBPK model with the posterior parameter estimates. These simulations indicated that the ratio of 50^{th} and 95^{th} percentile values of the area under the blood concentration vs time curve for MTBE and TBA was less than a factor of two. The parameter estimates and distributions developed in this study should be useful for characterizing population distributions of the internal dose of MTBE for risk assessment applications.

5.1. INTRODUCTION

Probabilistic and population modeling approaches are increasingly being explored for use in risk assessment. Monte Carlo simulation is commonly used to predict pharmacokinetic variability using population distributions of mechanistic determinants. Recent developments in Bayesian statistical approaches are particularly relevant for characterizing the population distribution of pharmacokinetic determinants and tissue dose of chemicals (for review, see e.g. Krishnan and Johanson 2005). In this methodology, prior knowledge is combined with information gained from new data in order to generate posterior distributions of pharmacokinetic determinants. The posterior distributions of the pharmacokinetic determinants are statistically defined by various sets of information that can eventually be applied with Monte Carlo simulation for variability analysis.

There have been several recent reports of the development of population distribution of tissue dose of chemicals using physiologically-based pharmacokinetic (PBPK) models and Markov Chain Monte Carlo (MCMC) simulation (Bois *et al.* 1996, Jonsson and Johanson 2001, Jonsson *et al.* 2001a, 2002). The MCMC method randomly samples distributed parameter values through many iterations until the convergence of these parameters, consistent with both new experimental data as well as prior distributions. Ultimately, the resulting converged estimates of the parameters can be used in a standard Monte Carlo simulation approach to characterize population pharmacokinetic variability. The recent advance in population modeling approaches such as Bayesian MCMC simulation has enabled the combination of past and currently available data for improving the PBPK models intended for application in risk assessment.

The objective of the present study was to conduct Bayesian analysis of the inhalation pharmacokinetics of methyl *tert*-butyl ether (MTBE) and its oxidative metabolite *tert*-butyl alcohol (TBA) in humans. The inhalation pharmacokinetics of MTBE, an oxygenated gasoline additive, has been extensively studied in animals and humans (Johanson *et al.* 1995, Borghoff *et al.* 1996, Rao and Ginseberg 1997, Nihlen *et al.* 1998,

Prah *et al.* 2004). Several authors have also developed PBPK models for MTBE in animals and humans (e.g., Borghoff *et al.* 1996, Rao and Ginserberg 1997, Krishnan et al. 2005). However, none had conducted a Bayesian analysis to facilitate computation of the magnitude of interindividual variability in the kinetics of MTBE and TBA in humans. In the present study, posterior distributions of sensitive parameters of the human PBPK model were calculated from experimental data collected in human volunteers (Nihlen *et al.* 1998) and these were then combined with prior knowledge of the physiological parameters to simulate the population pharmacokinetics of MTBE and TBA.

5.2. METHODS

5.2.1. PBPK model of MTBE and TBA

The MCMC simulation was accomplished using a previously validated human PBPK model for MTBE with its metabolite TBA (Krishnan et *al.* 2005; **Figure 5-1**). The PBPK model was developed to simulate the pharmacokinetics of MTBE and TBA in human volunteers performing light workload (50W). The tissue uptake of inhaled MTBE was described as a perfusion-limited process in the PBPK model whereas one-compartmental description was used to describe the kinetics of TBA (Krishnan et *al.* 2005). The values of organ perfusion used in the model reflected individuals at resting state or performing physical work.

5.2.2. Experimental data and Priors on PBPK model parameters

Previously published data on blood concentrations of MTBE and TBA in individuals exposed to 5-50 ppm of MTBE were retrieved (Nihlen *et al.* 1998). In this study, ten adult volunteers (age 23-51 years) were exposed during 2 hours to MTBE while performing an exercise on a bicycle (light workload corresponding to 50W). Blood concentrations were determined at pre-determined time intervals, both during and after exposure (upto 22 hr post-exposure for 25 ppm; upto 46 hr for 50 ppm). The Bayesian simulations were conducted using exposure levels where both MTBE and TBA were detected in blood (i.e., exposure concentrations of 25 and 50 ppm). The individual body

weights (70-90 kg) from the human exposure study were also specified during the MCMC simulations.

Information pertaining to the distributions of the various physiological, physicochemical parameters were obtained from the literature (**Table 5-1**). Prior distributions (shape, variability, and uncertainty) of blood flows and tissue volumes for the MCMC analysis were taken from the posterior values as described in Jonsson *et al.* (2001b). Prior distributions of partition coefficients were based on the the range of values from Nihlen *et al.* (1995). The distribution of the intrinsic clearance for MTBE and TBA as well as the volume of distribution of TBA were given large standard deviations (50% of mean) arbitrarily since prior population statistics was not available for these parameters. All parameter distributions were truncated at more than 3 times the standard deviations to provide the MCMC analysis some latitude in the estimation of posterior distributions.

5.2.3. Sensitivity analysis of the PBPK model

The sensitivity of the human PBPK model parameters toward the blood concentration of MTBE and TBA was evaluated in AcslXtreme® (Aegis Technologies). The time influence of the parameters was compiled under exposure condition described in Nihlen *et al.* (1998). Sensitivity coefficients were computed by means of the forward difference method as the amount of change in blood concentration related to 50% change of every parameter in the model. Sensitivity coefficients were assessed between MTBE blood concentration and the physiological parameters whereas TBA blood concentration was correlated to TBA volume of distribution, MTBE and TBA intrinsic clearance.

5.2.4. *Bayesian (MCMC) simulation*

The MTBE PBPK model with the prior information and experimental data was compiled in MCSim software (version 5.0.0; http://toxi.ineris.fr). Statistical analysis of the posterior estimates of the parameters was performed with BOA package for R and S-Plus (B. Smith, University of Iowa College of Public Health). The convergence of the sensitive parameters was obtained after 30,000 iterations. The convergence was analyzed

based on the posterior results of triplicate analyses, each with a different random seed value. An example of converging parameters is presented in **Figure 5-2**.

5.2.5. *Monte Carlo simulation*

Monte Carlo simulations with MCSim software were conducted using the PBPK model with the posterior parameter estimates to characterize interindividual variability of internal dose in a population exposed to MTBE. The interindividual variability factor was subsequently calculated as the ratio of the 95^{th} and 50^{th} percentile values for internal doses such as maximal blood concentration (C_{max}) or area under the blood concentration versus time curve (AUC).

5.3. RESULTS

5.3.1. *Sensitivity analysis*

The sensitivity of the key parameters of the PBPK model towards the arterial blood concentration of MTBE and TBA is depicted in **Figure 5-3**. MTBE blood concentration was most sensitive to changes in alveolar ventilation, cardiac output, MTBE hepatic clearance and to a lesser extent to MTBE blood:air partition coefficient. The impact of the sensitive parameters was most noticeable during the first 2 hours (i.e., exposure period). As one would anticipate, TBA blood concentration was markedly sensitive to MTBE clearance, TBA clearance, and TBA volume of distribution.

5.3.2. *Bayesian analysis*

The Bayesian simulation of the pharmacokinetics of MTBE and TBA was conducted by applying prior statistical distributions for the sensitive parameters of PBPK model. The posterior parameters acquired from the MCMC simulation of the MTBE/TBA PBPK model are listed in **Table 5-2**. The posterior estimates are compared with prior estimates for the various sensitive parameters of the PBPK model in **Figure 5-4.** In general, the posterior means of the PBPK determinants changed up to 20% from the prior values. The standard deviations of the posterior estimates for the MTBE-related parameters remained

high (geometric standard deviations above 1.50) compared to the prior values. The standard deviations associated with the posterior estimates of the TBA model were slightly less than those for the prior values. The standard deviation for each posterior estimate ranged from 1.0 to 1.5 whereas the prior uncertainty standard deviations were same for all the parameters (1.1).

MTBE and TBA blood concentrations were then simulated using the posterior estimates from the MCMC simulations. **Figure 5-5** depicts the correlation between the blood concentrations of MTBE and TBA obtained with the resulting model and the individual-specific experimental values. The dotted lines in these plots represent the confidence interval (95%). The confidence interval for MTBE and TBA blood concentrations deviate by about ± 1.5 and ± 5.0 μM, respectively, from the overall mean values. The correlations shown in **Figure 5-5** are based on blood concentrations for the entire group of volunteers, collected at various sampling times. The MCMC simulations and experimental blood concentrations of MTBE and TBA in two individuals are presented in **Figure5- 6**.

5.3.3. *Monte Carlo analysis*

Monte Carlo simulations of the PBPK model were generated using the posterior parameter estimates obtained with MCMC analysis. The simulations were conducted with MCSim using the posterior estimates for the parameters of MTBE and TBA models obtained in the present study along with physiological parameter distributions for a general population obtained from Thomas et al. (1996) and Jonsson and Johanson (2001). The 95% confidence interval of the distribution of PK profiles generated by the Monte Carlo simulations is illustrated in **Figure 5-7** along with the respective average population experimental data. The predicted peak MTBE blood concentration (at 2 hours) ranged from 4.6 to 11 μM for 25 ppm and from 9.3 to 22 μM for 50 ppm. The model simulated peak TBA blood concentration (at 5 hours) ranged from 2.5 to 9.0 μM for 25 ppm and from 5.0 to 18 μM for 50 ppm. The distribution of blood concentrations encompassed the experimental data of Nihlen *et al.* (1998) [average MTBE and TBA

concentrations were respectively 6.38 ± 0.64 and 7.01 ± 0.78 μM for 25 ppm exposure, and 11.66 ± 0.44 and 12.85 ± 0.99 μM for 50 ppm exposure].

The Monte Carlo simulation of MTBE and TBA blood concentration were then used to characterize the interindividual variability of the pharmacokinetics of MTBE and TBA for a given population. As presented in **Table5- 3**, the interindividual variability from the Monte Carlo simulation was computed as the ratio of the 95th and 50th percentiles of the peak blood concentration (C_{max}) and 24-hour area under the blood concentration *versus* time curve (AUC) for MTBE and TBA. The 95th and 50th percentiles of C_{max} varied by a factor of 1.5 and 1.9 for MTBE and TBA whereas AUC-based interindividual factor was 2.0 and 2.1, respectively for MTBE and TBA.

5.4. DISCUSSION

The Bayesian approach to PBPK modeling facilitates the use of all available information by combining prior knowledge with newly available data. In the present study, posterior estimates of distributions of chemical-specific parameters were obtained using MCMC simulations by combining prior estimates with kinetic data collected in healthy adults performing light workload. Since there is limited information on the population pharmacokinetics of MTBE and its metabolite TBA, the present work characterized the population distribution of input parameters for the MTBE-TBA PBPK model. The most sensitive determinants of the PBPK model were initially identified since the distributions of these parameters are most likely to affect the population distribution of the internal dose of MTBE and TBA.

The interindividual variability of the pharmacokinetics of MTBE and TBA was characterized for a population that includes more than the healthy adult volunteers described by Nihlen et al. (1998). The MCMC-refined PBPK parameter estimates reflect the adults represented by the prior data used in the Bayesian analysis. A robust population risk assessment would require the consideration of a broader range of

individuals, and variety of exposure scenarios than the ones described in Jonsson and Johanson (2001). Since the posterior parameter estimates based on healthy adult volunteers may only apply to a subset of a general population, the upper and lower limits of MTBE and TBA model parameters (clearances, volume of distribution and blood:air partition coefficient) for Monte Carlo simulations were specified as 3 times the posterior standard deviations. In addition, physiological values at rest and at work for a general population described in Thomas et al. (1996) were also introduced during the Monte Carlo simulations. Use of population distributions of physiological parameters along with the results of the present study facilitated the evaluation of the interindividual variability factor for the general population. The Monte Carlo simulations indicated that the interindividual variability factor for MTBE and TBA based on area under the blood concentration *vs* time curve was within a factor of two.

In the present study, along with the experimental data of Nihlen et al. (1998), two studies were used as sources of prior information for blood flow rates and blood/tissue:air partition coefficients of MTBE PBPK model. The priors on the blood flows for the PBPK model were based on posterior results from Jonsson et al. (2001b)'s study on the Bayesian pharmacokinetic modeling of methyl chloride. However, the upper and lower bounds were kept large for optimal fitting of simulated and experimental values. Based on Nihlen et al. (1996), it was assumed that the experimental variability of the partition coefficients was normally distributed.

The posterior estimates following the MCMC simulation reflect the likelihood of the precision and size of the experimental data and extent of prior information available (Kass and Wasserman 1996, Bernillon and Bois 2000). These were obtained from the convergence of the estimates after several iterations during the MCMC simulation. The posterior estimates are considered to have converged once comparable results were obtained after repeated simulations (Jonsson *et al.* 2001a). Convergence of the parameters was validated using a numerical test, namely Brooks, Gelman & Rubin Convergence Diagnostic, found in the BOA software package. The convergence diagnostics allows to determine whether the latter part of the chain of iterations from

MCMC simulation has converged to the same value when the simulations were repeated with a different initial value (Dodds and Vicini 2004). The sensitive determinants of the MTBE PBPK model were considered to have converged after 30,000 iterations since the Brooks, Gelman & Rubin Convergence Diagnostics confirmed that the posteriors from three simulations were comparable. The statistics from the last half of the combined posteriors of all three simulations served as the basis of input distributions for Monte Carlo simulation for the characterization of interindividual variability of the PK of MTBE in an adult population.

The MCMC simulation of MTBE and TBA was performed by introducing prior information on the most sensitive parameters of the PBPK model. The results of sensitivity analysis are comparable to other similar studies on MTBE. Licata et al. (2004) reported that at high level of exposure, the model was more sensitive to changes in the metabolic parameters and to a lesser extent towards MTBE blood:air partition coefficient, alveolar ventilation rate, cardiac output, fat:blood partition coefficient, and blood flow to fat. Although Bois et al. (1996) suggested that all model parameters should be introduced with prior information for the model to be properly fit in a full Bayesian sense, only the sensitive parameters of the PBPK were re-estimated during the MCMC simulations in the present study. MCMC simulation attempts (not shown) demonstrated that the posterior estimates generated with prior distribution on all the PPBK model parameters (including the less sensitive parameters) were equivalent to the results in **Table 5-2**. Therefore, Bayesian simulation of the sensitive parameters was sufficient to estimate posterior values on the basis of the experimental results from Nihlen et al (1998).

In conclusion, the MCMC PBPK simulation of MTBE illustrates that it is feasible to estimate chemical-specific parameters by combining prior knowledge with new pharmacokinetic measurements. The current work illustrates the application of a Bayesian PBPK modeling framework for population characterization of interindividual variability of MTBE. The posterior standard deviations and uncertainty of the PBPK model determinants obtained by the MCMC simulation were half as large as the mean values. Overall, for the risk assessment of volatile chemicals such as MTBE, posterior

estimates obtained by Bayesian PBPK simulations would satisfy the data requirements for the characterization of chemical-specific interindividual pharmacokinetic variability factor.

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5.5. REFERENCES

Bernillon, P., and Bois, F.Y. 2000. Statistical issues in toxicokinetic modeling: a bayesian perspective. *Environ. Health. Perspect.* 108 Suppl 5, 883-93.

Bois, F.Y., Jackson, E.T., Pekari, K., and Smith, M.T. 1996. Population toxicokinetics of benzene. *Environ. Health. Perspect.* 104 Suppl 6:1405-11.

Borghoff, S.J., Murphy, J.E., and Medinsky, M.A. 1996. Development of physiologically based pharmacokinetic model for methyl tertiary-butyl ether and tertiary-butanol in male Fisher-344 rats. *Fundam. Appl. Toxicol.* 30, 264-275.

Dodds, M.G., and Vicini, P. 2004. Assessing convergence of Markov chain Monte Carlo simulations in hierarchical Bayesian models for population pharmacokinetics. *Ann. Biomed. Eng.* 32, 1300-13.

Jarabek, A.M. 1995. The application of dosimetry models to identify key processes and parameters for default dose-response assessment approaches. *Toxicol. Lett.* 79, 171-84.

Johanson, G., Nihlen, A., and Lof, A. 1995. Toxicokinetics and acute effects of MTBE and ETBE in male volunteers. *Toxicol. Lett.* 82-83:713-8.

Jonsson, F., Bois, F.Y., and Johanson, G. 2001b. Assessing the reliability of PBPK models using data from methyl chloride-exposed, non-conjugating human subjects. *Arch. Toxicol.* 75, 189-199.

Jonsson, F., Bois, F.Y., and Johanson, G. 2001a. Physiologically based pharmacokinetic modeling of inhalation exposure of humans to dichloromethane during moderate to heavy exercise. *Toxicol. Sci.* 59, 209-18.

Jonsson, F., and Johanson, G. 2001. A Bayesian analysis of the influence of GSTT1 polymorphism on the cancer risk estimate for dichloromethane. *Toxicol. Appl. Pharmacol.* 174, 99–112.

Jonsson, F., and Johanson, G. 2002. Physiologically based modeling of the inhalation kinetics of styrene in humans using a bayesian population approach. *Toxicol. Appl. Pharmacol.* 179, 35-49.

Kass, R.E., and Wasserman, L. 1996. The selection of prior distribution by formal rules. *J. Am. Stat. Assoc.* 91, 1343-1370.

Krishnan, K., Nihlen, A., Ernstgard, L., and Johanson, G. 2005. Simulation of the inhalation pharmacokinetics of methyl tert-butyl ether in humans. In: J.G. Anderson and M. Katzper (Eds.), *Proceedings of the 2005 International Health Sciences Simulation Conference* (ISBN 1-56555-289-X). The Society for Computer Simulation International, San Diego, pp 105-110.

Krishnan, K., and Johanson, G. 2005. Physiologically-based pharmacokinetic and toxicokinetic models in cancer risk assessment. *J. Environ. Sci. Health.* 23, 31-53.

Licata, A.C., Dekant, W., Smith, C.E., and Borghoff, S.J. 2001. A physiologically based pharmacokinetic model for methyl tert-butyl ether in humans: implementing sensitivity and variability analyses. *Toxicol. Sci.* 62, 191-204.

Nihlen, A., Lof, A., and Johanson, G. 1995. Liquid/air partition coefficients of methyl and ethyl T-butyl ethers, T-amyl methyl ether, and T-butyl alcohol. *J. Expo. Anal. Environ. Epidemiol.* 5,573-82.

Nihlen, A., Lof, A., and Johanson, G. 1998. Experimental exposure to methyl tertiary-butyl ether. I. Toxicokinetics in humans. *Toxicol. Appl. Pharmacol.* 148,274-80.

Prah, J., Ashley, D., Blount, B., Case, M., Leavens, T., Pleil, J., and Cardinali, F. 2004. Dermal, oral, and inhalation pharmacokinetics of methyl tertiary butyl ether (MTBE) in human volunteers. *Toxicol. Sci.* 77,195-205.

Rao, H.V., and Ginsberg, GL. 1997. A physiologically-based pharmacokinetic model assessment of methyl t-butyl ether in goundwater for a bathing and showering determination. *Risk. Anal.* 17,583-598.

Thomas, R.S., Bigelow, P.L., Keefe, T.J., and Yang, R.S. 1996. Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. *Am. Ind. Hyg. Assoc. J.* 57, 23-32.

Table 5-1 Prior distribution of input parameters of the PBPK model for methyl *tert*-butyl ether (MTBE) and *tert*-butanol (TBA)

Parameter	Prior Distributions				
		Interindividual variability			Uncertainty
	Shape	Mean	Standard Deviation	Interval	Standard Deviation
Resting blood flows[1,2]					
Alveolar ventilation (L/hr/kg)	LogNormal	15.97	1.15	(1.0 - 60)	1.1
Cardiac output (L/hr/kg)	LogNormal	13.89	1.15	(1.0 - 60)	1.1
Additional work[1,2]					
Alveolar ventilation (L/hr/kg)	LogNormal	36.42	1.7	(1.0001 - 100)	1.1
Cardiac output (L/hr/kg)	LogNormal	9.53	1.7	(1.0001 - 100)	1.1
Chemical specific[2]					
MTBE clearance (L/hr)	LogNormal	100	1.6	(0.0001 - 1000)	1.1
MTBE blood:air partition coefficient[3]	Normal	17.7	2.48	(1.001 - 5.0)	0.24
TBA clearance (L/hr)	LogNormal	10	1.6	(0.0001 - 1000)	1.1
TBA volume of distribution (L)	LogNormal	100	1.5	(0.0001 - 1000)	1.1
Residual Error[3]					
MTBE	Normal	0.01	0.001		
TBA	Normal	0.1	0.01		

[1] Blood flows are scaled to the body weight as $Qt = Qtc \times BW^{0.74}$.
[2] Geometric mean and geometric standard deviation are designated for lognormal distributions.
[3] Normal distributions were defined with arithmetic mean and arithmetic standard deviation.
[4] Prior standard deviations and uncertainty values were taken from *Jonsson et al. (2001b)*. Mean MTBE and TBA clearances and TBA volume of distribution were from *Krishnan et al. (2005)*.

Table 5-2 Posterior estimates of the sensitive parameters of the MTBE PBPK model obtained with Markov Chain Monte Carlo simulation

Parameter	Posterior Estimates				
	Interindividual variability				Uncertainty
	Geometric Mean	Standard Deviation	Percentiles 5th	Percentiles 95th	Standard Deviation
Resting blood flows[1,2]					
Alveolar ventilation (L/hr/kg)	16.1	1.18	12.0	20.8	1.14
Cardiac output (L/hr/kg)	14.1	1.85	10.7	17.9	1.14
Additional work[1,2]					
Alveolar ventilation (L/hr/kg)	40.4	1.73	14.6	84.6	1.47
Cardiac output (L/hr/kg)	11.7	1.82	3.9	28.2	1.53
Chemical specific[2]					
MTBE clearance (L/hr)	119	1.60	37.5	257	1.46
MTBE blood:air partition coefficient[3]	15.1	2.50	13.7	16.5	1.04
TBA clearance (L/hr)	11.3	1.39	3.8	22.7	1.47
TBA volume of distribution (L)	121	1.26	54.9	251	1.49
Residual Error[3]					
MTBE	0.314				
TBA	1.637				

[1] Blood flows are scaled to the body weight as $Qt = Qtc \times BW^{0.74}$.
[2] Geometric means are designated for lognormal distributions.
[3] Normal distributions were defined with arithmetic means.

Table 5-3 **Percentile values of area under the venous blood concentration vs time curves (AUC) and maximal arterial blood concentration (C_{max}) of methyl *tert*-butyl ether (MTBE) and *tert*-butanol (TBA)**

	Percentiles			Interindividual Factor[3]
	5th	50th	95th	
		AUC[1] (mM.hr)		
MTBE				
25 ppm	15	24	47	2.0
50 ppm	29	48	95	2.0
TBA				
25 ppm	36	74	160	2.1
50 ppm	73	150	320	2.1
		C_{max}[2] (mM)		
MTBE				
25 ppm	4.6	7.3	11	1.5
50 ppm	9.3	15	22	1.5
TBA				
25 ppm	2.5	4.8	9.0	1.9
50 ppm	5.0	9.6	18	1.9

[1] AUCs were calculated for a 24-hour period (exposure duration: 2 hr, exposure concentration: 25-50 ppm of MTBE);
[2] C_{max} were obtained at 2 hours and at 5 hours of exposure for MTBE and TBA.
[3] The interindividual variability factor was calculated as the ratio of 95th percentile value over the 50th value.

Figure Legends

Figure 5-1 Human PBPK model of methyl *tert*-butyl ether (MTBE) and *tert*-butanol (TBA) (Krishnan *et al.* 2005).

Figure 5-2 Convergence of methyl *tert*-butyl ether (MTBE) and *tert*-butanol (TBA) clearance posteriors after 30,000 iterations of the Markov Chain Monte Carlo (MCMC) simulation. Three chains of simulations are depicted for MTBE hepatic clearance and TBA hepatic clearance.

Figure 5-3 Sensitivity coefficients of the methyl *tert*-butyl ether (MTBE) and *tert*-butanol (TBA) PBPK model parameters against the simulated arterial blood concentrations. The most sensitive parameters toward MTBE blood concentration are alveolar ventilation (Qp), cardiac output (Qc), MTBE hepatic clearance (Cl_{mtbe}), MTBE blood:air partition coefficient and body weight. Whereas, TBA blood concentration is sensitive to MTBE hepatic clearance, TBA clearance (Cl_{tba}) and TBA volume of distribution (Vd_{tba}).

Figure 5-4 Percent change of means and standard deviations between prior and posterior of the sensitive parameters for the Markov Chain Monte Carlo simulation of methyl *tert*-butyl ether (MTBE) and *tert*-butanol (TBA) PBPK model. The most sensitive parameters toward MTBE blood concentration are alveolar ventilation (Qp), cardiac output (Qc), MTBE hepatic clearance (Cl_{mtbe}), MTBE blood:air partition coefficient and body weight. Whereas, TBA blood concentration is sensitive towards MTBE hepatic clearance, TBA clearance (Cl_{tba}) and TBA volume of distribution (Vd_{tba}).

Figure 5-5 Comparison of predicted and experimental distributions of methyl *tert*-butyl ether (MTBE) and *tert*-butanol (TBA) blood concentrations. The confidence intervals ($\alpha = 0.05$) of predicted blood concentration are shown in dotted lines.

Figure 5-6 Markov Chain Monte Carlo (MCMC) simulations and experimental data of methyl *tert*-butyl ether (MTBE) and *tert*-butanol (TBA) in two individuals. Open and filled symbols represent respectively exposure to 50 ppm and 25 ppm MTBE. Data from Nihlen *et al.* (1998).

Figure 5-7 Comparison of Monte Carlo simulations (with 5^{th} and 95^{th} percentile bounds) based on Bayesian posteriors of the present study and physiological parameters from Thomas et al. (1996) with the experimental data for methyl *tert*-butyl ether (MTBE) (A: 25 ppm; C: 50 ppm) and *tert*-butanol (TBA) (B: 25 ppm; D: 50 ppm) collected in human volunteers (Nihlen *et al.* 1998).

Figure 5-1

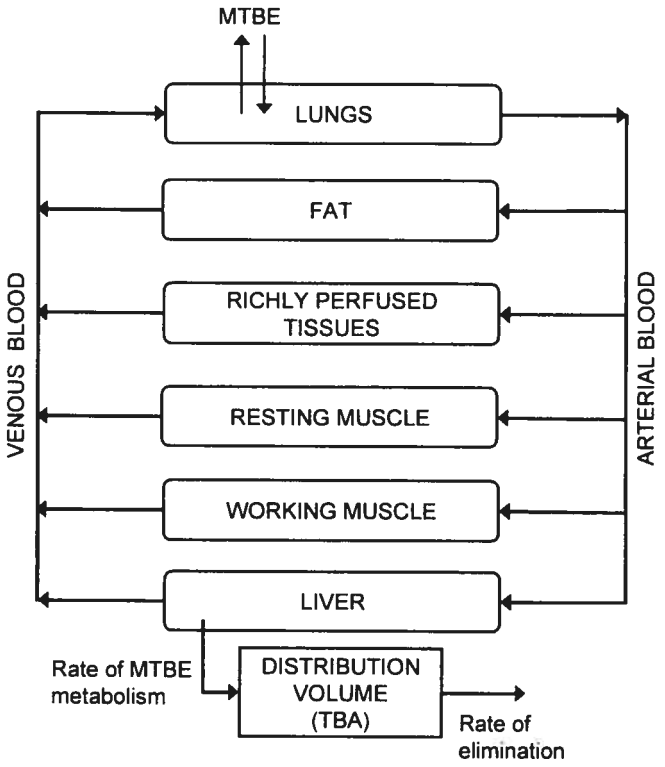

MTBE
LUNGS
FAT
RICHLY PERFUSED TISSUES
RESTING MUSCLE
WORKING MUSCLE
LIVER
VENOUS BLOOD
ARTERIAL BLOOD
Rate of MTBE metabolism
DISTRIBUTION VOLUME (TBA)
Rate of elimination

Figure 5-2

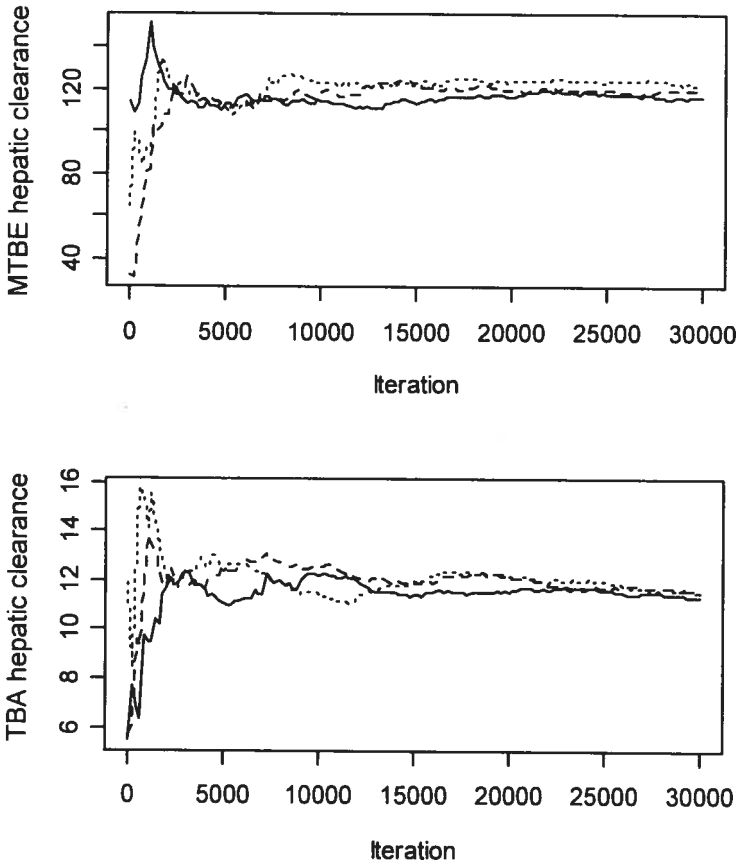

MTBE hepatic clearance
40
80
120
0
5000
10000
15000
20000
25000
30000
Iteration
TBA hepatic clearance
6
8
10
12
14
16
0
5000
10000
15000
20000
25000
30000
Iteration

Figure 5-3

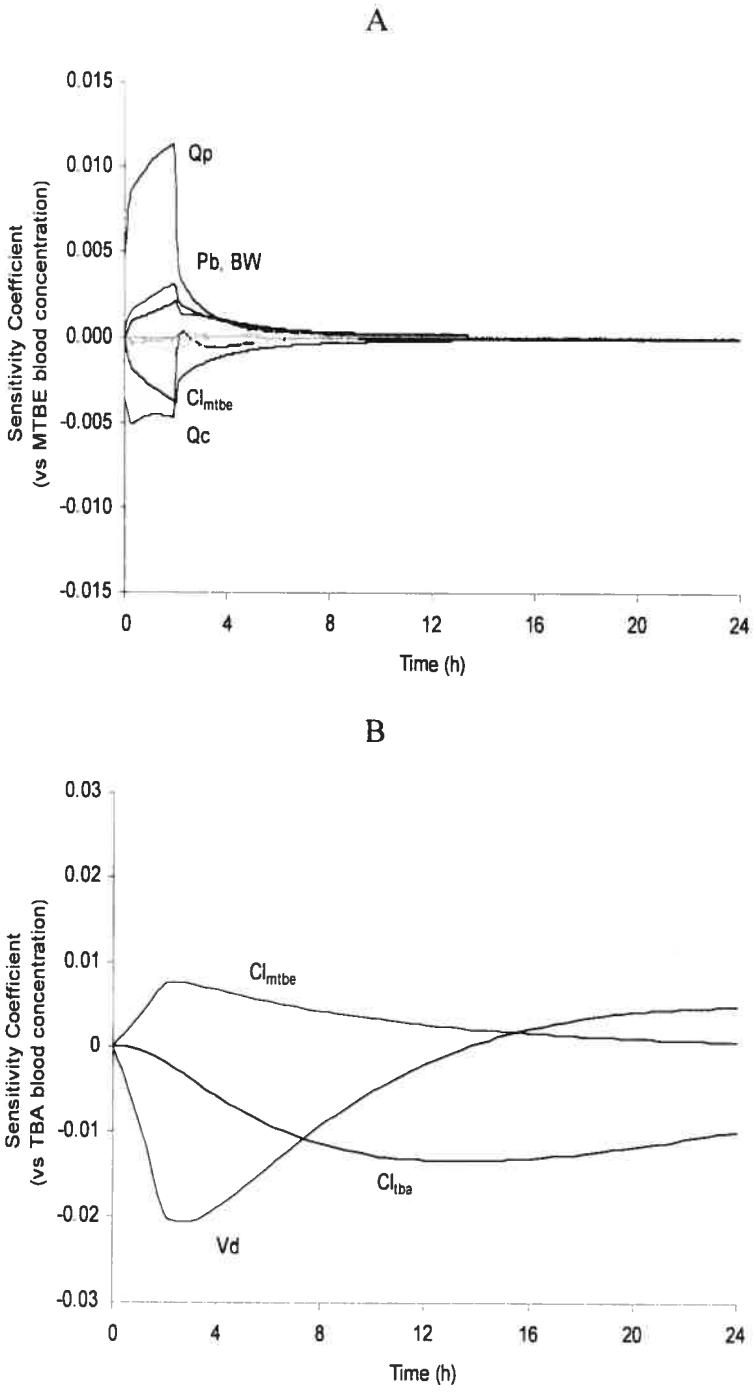

A
0.015
0.010
0.005
0.000
-0.005
-0.010
-0.015
Sensitivity Coefficient
(vs MTBE blood concentration)
Qp
Pb, BW
Clmtbe
Qc
0
4
8
12
16
20
24
Time (h)
B
0.03
0.02
0.01
0
-0.01
-0.02
-0.03
Sensitivity Coefficient
(vs TBA blood concentration)
Clmtbe
Cltba
Vd
0
4
8
12
16
20
24
Time (h)

Figure 5-4

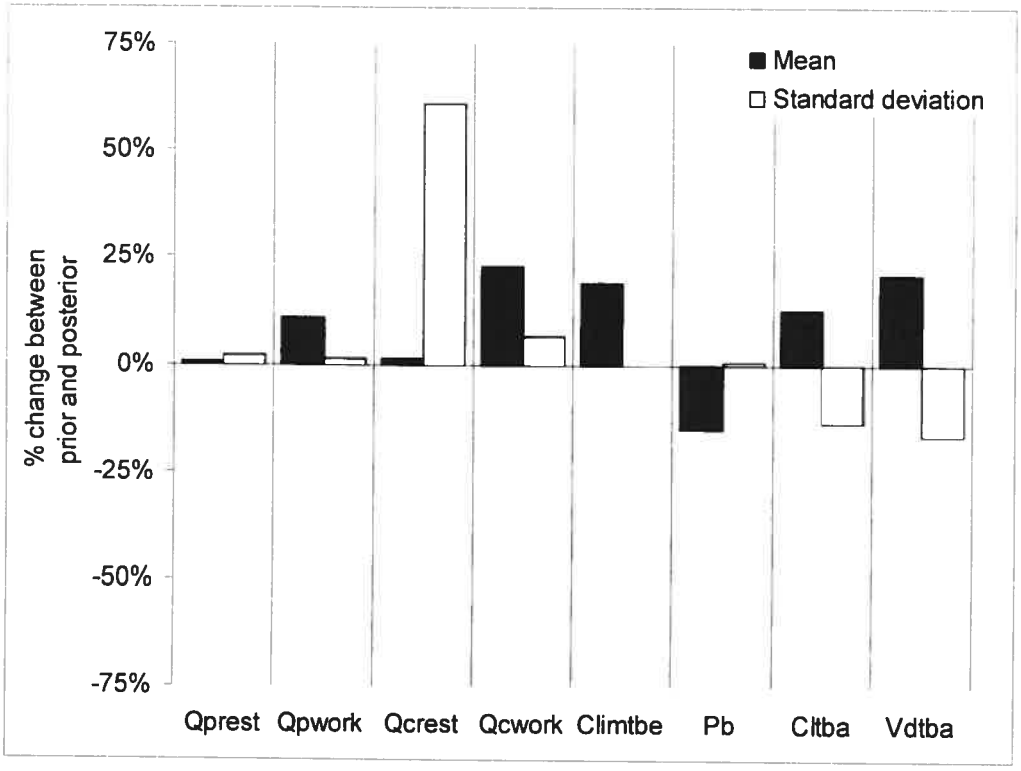

75%
50%
25%
0%
-25%
-50%
-75%
% change between prior and posterior
Mean
Standard deviation
Qprest
Qpwork
Qcrest
Qcwork
Climtbe
Pb
Cltba
Vdtba

Figure 5-5

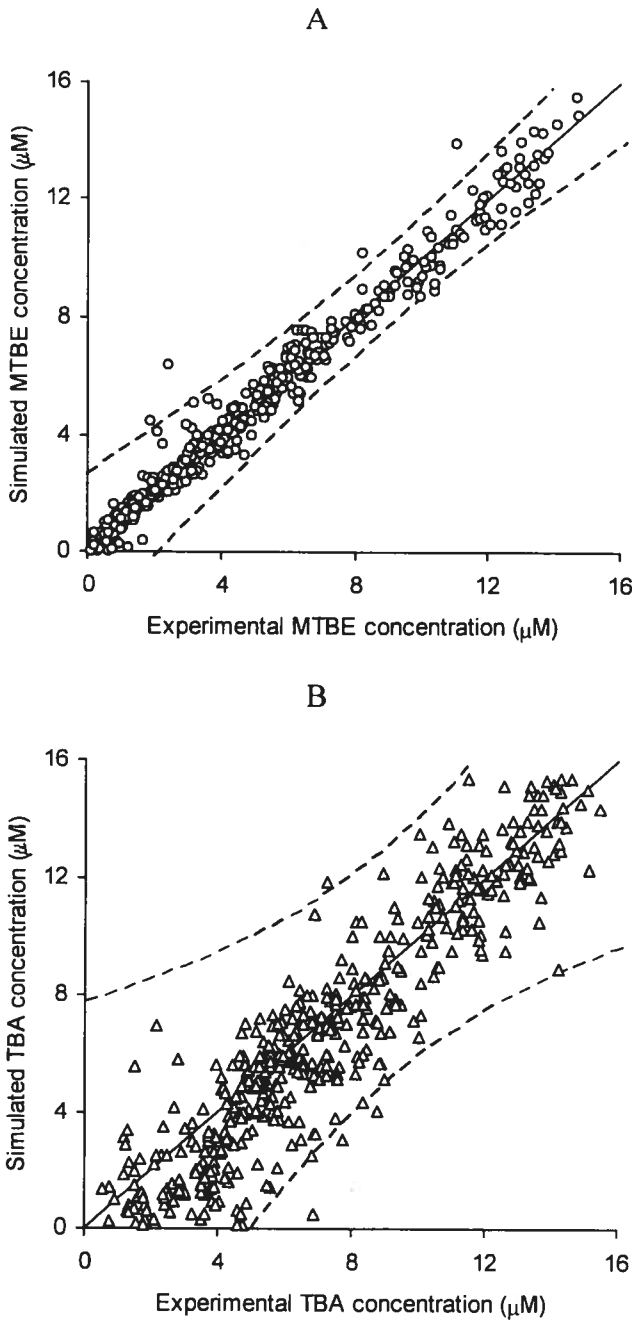

A
Simulated MTBE concentration (μM)
Experimental MTBE concentration (μM)
0
4
8
12
16
B
Simulated TBA concentration (μM)
Experimental TBA concentration (μM)
0
4
8
12
16

Figure 5-6

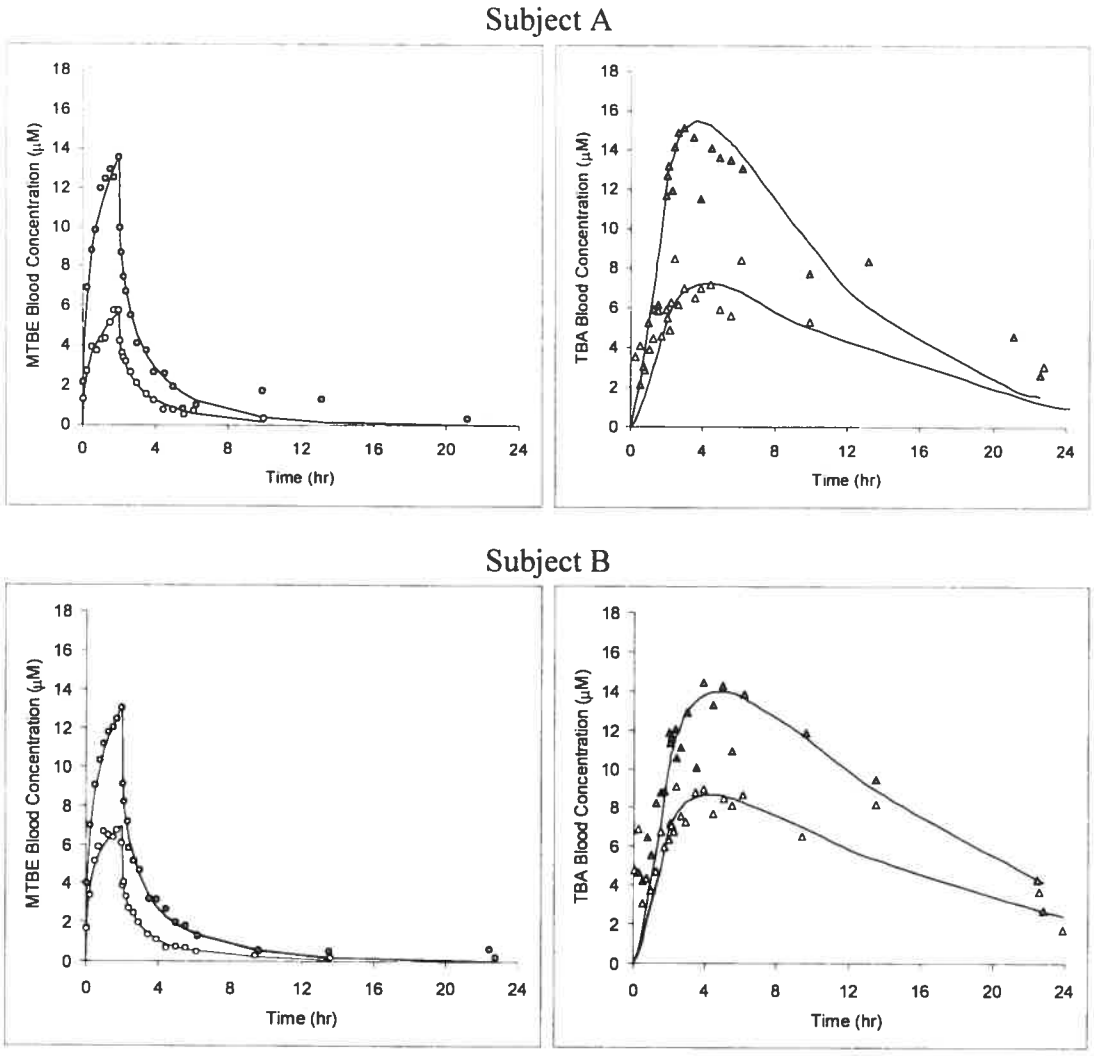
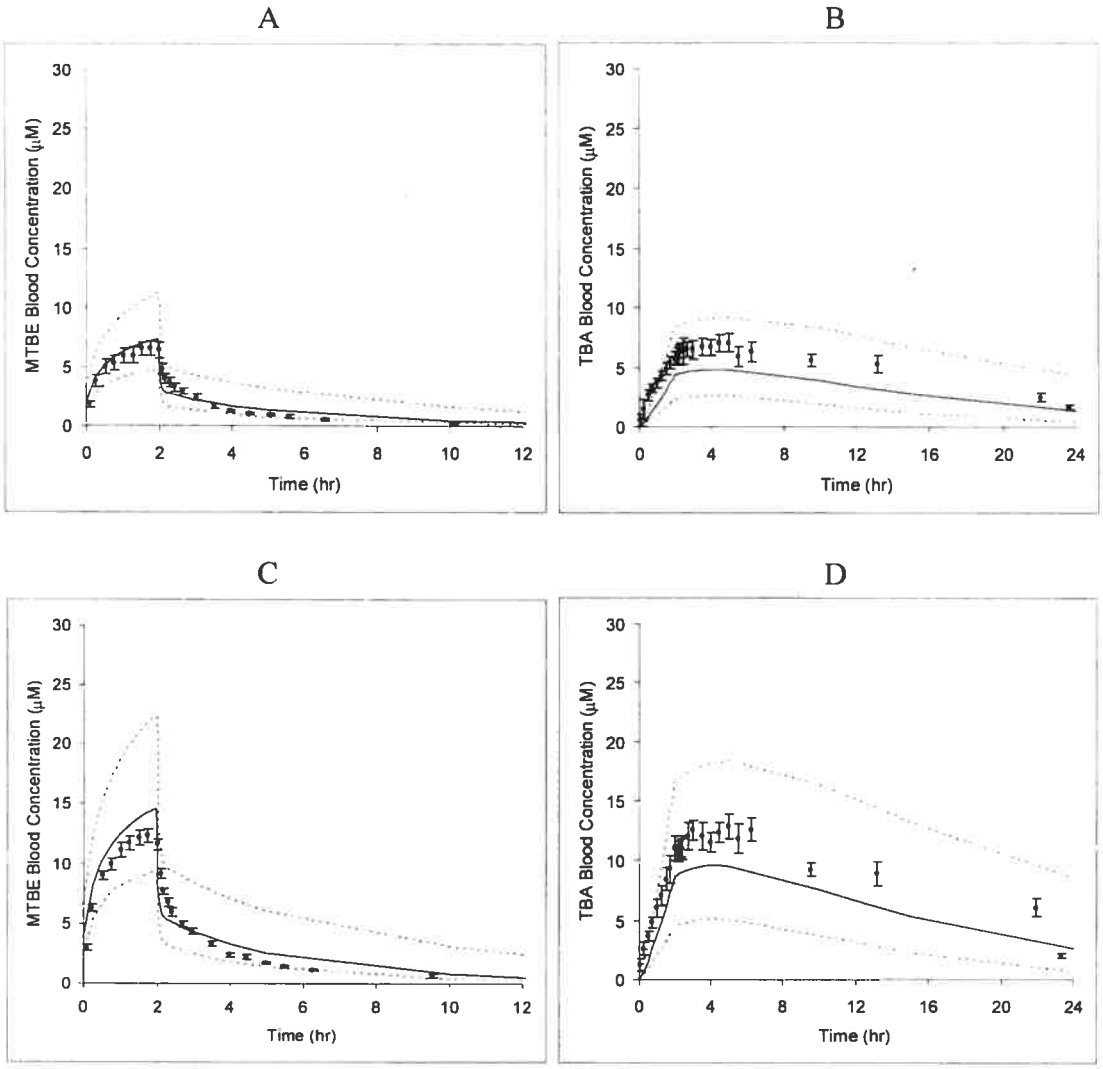

Subject A
MTBE Blood Concentration (μM)
TBA Blood Concentration (μM)
Time (hr)
Subject B
MTBE Blood Concentration (μM)
TBA Blood Concentration (μM)
Time (hr)

Figure 5-7


A
B
C
D
MTBE Blood Concentration (μM)
TBA Blood Concentration (μM)
Time (hr)
0
5
10
15
20
25
30
0
2
4
6
8
10
12
16
20
24

CHAPITRE VI

6. ARTICLE IV

An approach for estimating population-specific inter-individual variability factor.
Nong, A., and Krishnan, K.

Nong, A., and Krishnan, K. 2006. An approach for estimating population-specific inter-individual variability factor. (à soumettre).

An approach for estimating population-specific inter-individual variability factor

Nong, A., and Krishnan, K.
Groupe de Recherche Interdisciplinaire en Santé (GRIS),
Université de Montréal, Montreal, QC, Canada.

Correspondance to :
Kannan Krishnan, Département de Santé Environnementale et Santé au Travail,
Université de Montréal, Montreal, QC, Canada. [redacted]

ABSTRACT

Interindividual variability factor (IVF) used in non-cancer risk assessments corresponds either to a default value (10) or to the value based on human data or model simulations. The currently available modeling approaches facilitate the determination of the pharmacokinetic component of IVF (IVF-PK) using information on population distributions of input parameters. The resulting IVF-PK is applicable for the population for which parameter distribution was obtained and not necessarily to other populations, particularly if the composition of populations is not identical - in terms of specific sub-groups such as children and elderly. The objective of the current study was therefore to develop a methodology to estimate population-specific IVF-PK on the basis of demographic data in terms of specific sub-populations (male and female adult, children, elderly and pregnant women; age range: birth to 90 years). Using Statistics Canada's most recent census for the metropolitan region of Montreal and the arctic region of Nunavik, individual-specific estimates of PK determinants were obtained with P^3M version 2.0 (Linea, Inc) and used as a basis to develop population-specific distributions for input parameters of PBPK models. Sample simulations were conducted for inhalation pharmacokinetics of toluene (at 1 ppm for 24 hr) in both populations. The comparison of the 24-hr area under the venous blood concentration versus time curve of toluene for the population of Montreal and Nunavik revealed that the values for many sub-populations were similar to adults, with an IVK-PK less than 1.5. Meanwhile, children and pregnant women had IVF-PK greater than 2.0 when compared to median population or adult blood concentrations. The modeling approach also allowed the estimation of the influence of the size of the various sub-populations on the magnitude of IVF-PK. This population-specific modeling approach uniquely facilitates the estimation of IVF-PK depending upon the nature and extent of available data on the chemical as well as the composition of exposed population.

6.1 INTRODUCTION

Interindividual variability factor (IVF) used in non-cancer risk assessments corresponds to the default value (10) when information pertaining to population differences is insufficient. The default value can also be substituted with a chemical-specific adjustment factor derived from experimental data or model simulations (IPCS 2001). Currently available modeling approaches facilitate the determination of the magnitude of the pharmacokinetic (PK) component of IVF (IVF-PK) using information on population distributions of input parameters. Population distribution of input parameters are introduced into a physiological model to simulate the magnitude of interindividual variability in pharmacokinetics. Such an approach has been applied to volatile organic chemicals (VOCs) for which IVF-PK was derived from distributions of internal doses obtained using Monte Carlo simulations of physiologically-based pharmacokinetic (PBPK) models (e.g., Droz *et al.* 1989, Portier and Kaplan 1989, Gearhart *et al.* 1993, Thomas *et al.* 1996). The resulting IVF-PK is applicable for the specific population for which parameter distribution was obtained. However, the Monte Carlo simulation results for one study population may not necessarily reflect the distribution in other types of populations, particularly if the composition of populations is not identical - in terms of specific sub-groups such as children, elderly and pregnant women.

Age-specific physiological differences have previously been accounted for in PBPK models (Clewell *et al.* 1999, Price *et al.* 2003a). Physical growth and as well as age-related changes in tissue volumes, tissue blood flow rates and metabolizing systems are known to influence the kinetics of chemicals, resulting in potential interindividual differences in internal doses (e.g., Lipscomb *et al.* 2003, Nong *et al.* 2006). Similarly, physiological and metabolic changes in other sub-populations such as the elderly and pregnant women might also lead to changes in internal dose of chemicals compared to typical adult. The population-specific distributions of various pharmacokinetic determinants may be evaluated and included within PBPK models to compute the magnitude of population-specific IVF-PK but this is yet to be attampted.

The objective of the current study was therefore to develop a methodology, based on PBPK modeling, to estimate population-specific IVF-PK as a function of demographic data in terms of specific sub-populations. Simulation of internal dose distribution of a volatile organic chemical such as toluene was generated using latest (2001) demographic census data for two cities of different population subgroup proportion, the greater metropolitan region of Montreal and artic region of Nunavik in the province of Quebec in Canada (**Figure 6-1**). Population subgroups were categorized into defined demographic age groups (neonate and toddler, child and adolescent, adults, late adulthood, elderly and pregnant women). Sub-population specific distribution of body weights, tissue volumes, tissue blood flows and hepatic enzyme concentration were specified within toluene PBPK model. IVF-PKs were then derived from the Monte Carlo simulations of internal dose in each subgroup as well as the entire general population.

6.2 METHODS

6.2.1 Population-specific PBPK modeling

The human PBPK model for toluene developed and validated by Tardif *et al.* (1995) was adopted for use in the present study. This model described inhalation exposure and accounted for hepatic metabolism by CYP2E1. This PBPK model was modified to include fetal-placental exchange in pregnant women as shown in **Figure 6-2**. The PBPK model structure and exposure conditions remained the same for all subgroups so that interindividual variability was characterized solely on the physiological and metabolic differences in each subgroup.

The PBPK model was then used to simulate the population distribution of blood concentrations of inhaled toluene in the two regions (Montreal vs Nunavik) according to the size of each subgroup and the statistical distributions of model determinants. The demographic information was taken from the latest available census data whereas the

model parameters were based on literature values and large scale survey figures as detailed below.

6.2.2 Canadian population statistics

The population statistics for the greater metropolitan Montreal area and the Nunavik region were obtained from 2001 census of Statistics Canada. These two Canadian populations had quite different demographic composition, in tems of neonate and toddler (0 to 4 years), child and adolescent (5 to 19 years), adult (20 -44), late adulthood (45 to 64 years), elderly (65 to 90 years) and pregnant women (14 to 45 years) as seen in **Table 6-1**. The metropolitan Montreal area consists of several large municipalities as well as the city of Montreal (over 3 million people), comprised mostly of an aging population with more than half aged between 20 to 64 years. On the other hand, the arctic area of Nunavik consisted of 11 Inuit villages, with a relatively young population of about 10,000 individuals (about half the population aged less than 20 years). The PBPK model simulations were based on Monte Carlo sampling founded on probability density of the various sub-populations reflecting the demographic data.

While demographic data for all population subgroups were defined by age, the size of the pregnancy group, in the present study, was calculated on the basis of number of women belonging to the child-bearing age (i.e., 14 to 44 years) and the pregnancy rate for the province of Quebec published by Statistics Canada. Although physiological information for the other age subgroups was more readily available, many biological attributes for the pregnant women were generalized as explained in the following section.

6.2.3 Population specific physiological and metabolic distribution

For each population subgroups of Montreal and Nunavik, statistical distributions of parameters of toluene PBPK model were introduced. The subject-specific estimates of pharmacokinetic determinants based on the size of each age sub-population were obtained with P^3M version 1.2 (Linea Inc, Cape Elizabeth, ME) as seen in **Table 6-2**. The distribution of pharmacokinetic determinants from the P^3M software were established on the basis of values from the National Health and Nutrition Examination

Survey (NHANES III) for adult (men and women), children, and elderly (Price *et al.* 2003b).

The distributions of physiological and metabolic parameters in pregnant women were based on International Commission on Radiological Protection (ICRP, 2002) and Clewell *et al.* (1999). As listed in **Table 6-3**, the body weights, tissue volumes and tissue blood flows were based on values for the late term of pregnancy. Metabolic activity for all the subgroups was defined as a function of liver cytochrome 2E1 (CYP2E1) protein content. The age-related distributions of liver CYP2E were described by Lipscomb *et al.* (2003) for adults and elderly as well as by Jonshrud *et al.* (2003) for children and neonates. Age specific clearance based on CYP2E1 content were performed as per Nong *et al.* (2006).

Blood:air and tissue:blood partition coefficients of toluene were assumed to the same for all individuals, due to lack of evidence regarding their age-dependency. The partition coefficients are presented in **Table 6-4**. Fetal and placental partition coefficients were not available in humans and were estimated on the basis of measurements in pregnant rat (Ghantous and Danielsson 1986).

Sub-population specific distributions of body weights, tissue volumes, blood flows and liver enzyme content, and partition coefficients were then specified within a PBPK model for toluene (Tardif *et al.* 1995). The PBPK model was solved in Microsoft® Excel according to Haddad *et al.* (1996). Using the spreadsheet program, toluene blood concentrations of every subgroup were calculated.

6.2.4 Monte Carlo simulation and statistical analysis

Monte Carlo pharmacokinetic simulations for continuous inhalation exposure of toluene (at 1 ppm for 24 hr) based on the demographic size of different sub-populations were then obtained using Crystal Ball 7 (Decisioneering®, Denver, CO). Parameter distributions were specified for each population subgroup (**Tables 6-2 to 6-4**). The number of iterative runs produced by the Monte Carlo simulations was in accordance with the population subgroups size presented in **Table 6-1**.

Areas under the blood concentration *vs* time (AUC) were compared among the population subgroups for computing IVF-PK as the ratio of 95th percentile value over 50th percentile value (IPCS 2001). The 95th percentile for AUC in each subgroup was divided by the over 50th percentile from the AUC distribution in either the adults or the entire population.

During the Monte Carlo simulations, sensitivity of the PBPK model determinants were also calculated. Sensitivity ratios were calculated as contribution of variance of AUC by a single PBPK model parameter. The sensitivity ratios were calculated after 10,000 Monte Carlo iterations for each subgroup.

6.3 RESULTS

6.3.1 Population-specific PK distribution

Simulations of the kinetics of toluene in each subgroup of the population of greater metropolitan Montreal area and the arctic Nunavik region were obtained for daily inhalation exposure to toluene (at 1 ppm for 24 hr) using the PBPK model and the available population statistics. The distributions of simulated toluene area under the curve versus blood concentrations (AUC) for both Canadian regions are presented in **Figures 6-4 and 6-5**. The AUC distributions for the entire population of Montreal and Nunavik ranged from 0.1 to 1.0 mg/L.hr. The internal dose distributions illustrate the importance of physiological parameters and size of each subgroup in each population.

The overall distribution of $AUC_{toluene}$ for the population of Montreal is shown in **Figure 6-4** where the contribution of each age subgroup is also displayed. The average AUC as well as the standard deviation for the entire simulated population of Montreal was 0.29 ± 0.16 mg/L.hr. Adults, late adulthood and elderly subgroups which represent 75% of the whole population had an overall average AUC of 0.22 mg/L.hr (SD: 0.12 mg/L.hr). However, the "neonate to adolescent" groups had an average AUC of 0.42 mg/L.hr (SD:

0.12 mg/L.hr). Pregnant women were predicted to have an average and standard deviation AUC of 0.51 ± 0.11 mg/L.hr. For the present case, physiological and metabolic differences due to pregnant women and children which correspond to 25% of the population of Montreal would appear to have some considerable effect on the distribution of AUC compared to adults and elderly.

The AUC distribution for toluene in the general population of Nunavik and in each subgroup had some similarity with the results obtained for Montreal (**Figure 6-5**). The average and standard deviations of AUC for the entire simulated population of Nunavik was 0.31 ± 0.16 mg/L.hr. Adults and elderly, representing half the Nunavik's population, had a simulated average AUC of 0.22 (SD: 0.10) mg/L.hr. The other half of the Nunavik population consisting of children (neonates to adolescent) and pregnant women had an average AUC of 0.42 mg/L.hr (SD: 0.12 mg/L.hr) and 0.51 mg/L.hr (SD: 0.11 mg/L.hr), respectively. Consistent with the AUC distribution for Montreal, adults to elderly were in the left-hand side of the curve whereas children and pregnant women werem more to to the right (i.e., with higher AUCs). Despite the difference in the size and proportions of the various subgroups between Montreal and Nunavik, the AUC averages and standard deviations were quite similar.

6.3.2 IVF-PK analysis

Pharmacokinetic interindividual variability factor (IVF-PK) determined from the simulated distributions of $AUC_{toluene}$ for both Canadian regions are presented in **Tables 6-5 and 6-6**. In the case of Montreal region, for each population subgroup, the 95th percentile was compared against the entire population median (0.27 mg/L.hr) or the adult median (0.21 mg/L.hr) (**Table 6-5**). As a result, an IVF-PK greater than 2.0 for the entire population was obtained when the 95th percentile for the entire population (0.58 mg/L.hr) was divided by the population or adult median AUC. Matured subgroups (adults to elderly; age 20 to 90 years) had IVF-PKs of less than 1.5 since their 95th percentile AUCs (0.33, 0.32, 0.29 mg/L.hr) were less than that for the entire population. However, the 95th percentile for younger subgroups (0-19 years) and pregnant women (14-45 years) were comparable to that of the entire population (0.60, 0.60, 0.70 mg/L.hr). Hence, the IVF-

PKs for children and pregnant women were greater than 2.5 and 3.0 respectively (compared to the mean AUC values in adults). In the present case, the pharmacokinetic interindividual variability of toluene in the entire population was due to physiological and metabolic differences due to neonates and toddlers, children and adolescents as well as pregnant women.

Toluene IVF-PK estimates for the simulated population of Nunavik were similar to the values from the Montreal population. As seen in **Table 6-6**, each population subgroups 95th percentile AUC was compared against the entire population median (0.27 mg/L.hr) or the adult median (0.22 mg/L.hr). The entire population's IVF-PK was also more than 2.0 when the population 95th percentile AUC (0.57 mg/L.hr) was used. Older subgroups such as adults and elderly had an IVF-PK less than 1.5 where their 95th percentile AUC ranged from 0.28 to 0.33 mg/L.hr. Younger subgroups and pregnant women had IVF-PKs ranging from 2.2 to 3.1 since their 95th percentile AUC (0.60 to 0.67 mg/L.hr) was greater than the other subgroups. As was the situation for Montreal, the pharmacokinetic interindividual variability of toluene for the entire population Nunavik was also explained by physiological and metabolic differences young subgroups and pregnant women.

6.3.3 Sensitivity analysis

The sensitivity of the PBPK model parameters towards the AUC was analyzed for each population subgroup (**Figure 6-6**). This analysis indicated that certain population subgroup specific parameters (body weight, fat blood flow, liver blood flow, and liver CYP2E1 concentration) were most effective at affecting the interindividual variability of AUC in the population.

6.4 DISCUSSION

An interindividual variability factor of 10 is conventionally used in the risk assessment of inhaled and ingested contaminants. This composite factor of 10 is assumed to account for pharmacokinetic and pharmacodynamic differences among individuals of a given

population. This composite factor has been subdivided into factors of 3.2. Thus, the default IVF-PK of 3.2 is assumed to be sufficient to cover the interindividual difference in internal dose among the various individuals (or sub-groups) of a population. However, when the composition of the population changes, the distribution of internal dose or more specifically the magnitude of the factor calculated as the ratio of the 95^{th} and 50^{th} percentiles might be different. The present study challenged the current approach of using a population-indifferent value of IVF-PK. Because of biological changes occurring during growth, pregnancy and aging, the population pharmacokinetics might be actually more heterogeneous compared to the typical, average individual. Based on available information, the present study attempted the estimation of subpopulation-specific influence on the pharmacokinetic determinants and magnitude of IVF-PK. The assumption that demography influences the magnitude of IVF-PK has been verified for the first time in this study using population-specific distributions of parameters and modeling strategy. Using toluene as the model substrate, the present study has shown that the magnitude of IVF-PK is comparable between the two populations investigated, i.e., Montreal and Nunavik.

Several previous studies have used population distributions of input parameters for modeling the internal dose of chemicals, with a PBPK-Monte Carlo simulation approach (e.g., Droz *et al.* 1989, Portier and Kaplan 1989, Gearhart *et al.* 1993, Thomas *et al.* 1996). However, these studies all used (i) distributions of parameters for an adult populaton, and (ii) applied the modeling approach to estimate IVF-PK regarldless of the composition of the population. Even though the magnitude of IVF-PK is not very different between the two populations investigated in the present study, the approach developed uniquely took into account the demographic data in the process of characterization of internal dosimetry and IVF-PK for toluene.

Among the different subpopulations modeled, children (including neonates and adolescent) and pregnant women had 95^{th} percentile values that was comparable to that for the whole population of Montreal and Nunavik. This implies that these subpopulations influenced the magnitude of the IVF-PK in both populations. The known

physiological and metabolic differences between neonates and adults or pregnant women and adults, essentially translates into an impact on the magnitude of TVF-PK. Immaturity of many biological systems in the child leads to higher internal doses of parent chemicals (Price *et al.* 2003a, Ginsberg *et al.* 2004, Nong *et al.* 2006). For example, CYP2E1 is very low or nil in neonates approaching adult level (about 57 pmol/mg microsomal protein) after 3 months to a year (Vieira *et al.* 1996, Cresteil 1998, Johnsrud *et al.* 2003, Lipscomb *et al.* 2003). Similarly, physiological alterations during pregnancy have also distinct bearing on the pharmacokinetics of xenobiotics. Many physiological and metabolic changes associated with pregnancy can lead to increase or decrease in the internal dose of xenobiotics in the mother (Mattison *et al.* 1991, O'Flaherty 1998, Little 1999, ICRP 2002, Syme *et al.* 2004). The proportion of children and pregnant women differed between Montreal and Nunavik populations were markedly different; however the estimated magnitude of IVF-PK for toluene in ; both populations was quite similar. The IVF-PKs for the whole population (between 2.1 and 2.4) changed by less than 15% when the fraction of the sensitive subpopulations varied from 25% in Montreal to 50% in Nunavik. In fact, if the population size of the infants or pregnant women were to double, the IVF-PK calculated with the 95^{th} percentile of AUC for the entire population of Montreal or Nunavik would have increased by less than 15%. Even though the sensitivity of the subpopulation size is not directly comparable to that of pharmacokinetic determinants, it is important to realise that the number of individuals exhibiting high internal dose levels would be different between Montreal and Nunavik at the same prevalence. The sensitivity analysis provided further insight on population-dependency of interindividual variability in terms of pharmacokinetic determinants of specific subpopulations (e.g., CYP content in neonates, liver blood flow in pregnant women). The sensitive determinants in specific subpopulations identified in Figure 6-6 influence the 24-hr AUC and as well as the magnitude of IVF-PK for toluene calculated for a given population.

The comparison of 24-hr area under the venous blood concentration vs time curves of toluene among the sub-populations of the Montreal and Nunavik area revealed that the IVK-PK is within the default value (3.16 or $10^{1/2}$). The magnitude of variability of

internal dose in a population depends upon characteristics of the chemical, mechanisms of pharmacokinetics as well as characteristics of the exposed population. The magnitude of IVF-PK estimated in the present study would be similar for other chemicals that exhibit similar blood:air partition coefficient, tissue:blood partition coefficients as well as hepatic clearance, for which the key determinants are blood and tissue contents of lipid and heptic CYP2E1 activity. When there is qualitative or quantitative difference in the mechanistic determinants of internal dose (e.g., hepatic vs renal clearance), there is a greater possibility that IVF-PK for chemicals would also be different for the same population. However, the use of the current approach is scientifically sound compared to the conventional approach of using an IVF-PK of 3.16 for all chemicals regardless of the nature of the toxic moiety, composition of the population and the characteristics of the chemical.

Overall, this population-specific modeling approach uniquely facilitates the estimation of IVF-PK depending upon the nature and extent of available data on the chemical as well as the target population. The present case study was applied to characterize the interindividual variability of internal dose of inhaled toluene in the populations of Montreal and Nunavik. The application of this approach with other contaminants, exhibiting extreme values of partition coefficients and metabolism rates, should lead to the identification of those populations for which the default factors will hold good and others that may not be adequately protected by the default factor. The proposed PBPK-Monte Carlo modeling approach is scientifically sound for the characterization of population-specific IVF-PK based on simulations of variability in internal dose of chemicals.

6.5 REFERENCES

Bogdanffy, M.S., and Jarabek, A.M. 1995. Understanding mechanisms of inhaled toxicants: implications for replacing default factors with chemical-specific data. Toxicol Lett. 82-83, 919-32.

Clewell, H.J., Gearhart, J.M., Gentry, P.R., Covington, T.R., VanLandingham, C.B., Crump, K.S., and Shipp, A.M. 1999. Evaluation of the uncertainty in an oral reference dose for methylmercury due to interindividual variability in pharmacokinetics. Risk Anal. 19, 547-58.

Clewell, H.J., Teeguarden, J., McDonald, T., Sarangapani, R., Lawrence, G., Covington, T., Gentry, R., and Shipp, A. (2002). Review and evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. Crit Rev Toxicol. 32, 329-89.

Corley, R.A., Mast, T.J., Carney, E.W., Rogers, J.M., and Daston, G.P. 2003. Evaluation of physiologically based models of pregnancy and lactation for their application in children's health risk assessments. Crit Rev Toxicol. 33, 137-211.

Cresteil, T. 1998. Onset of xenobiotic metabolism in children: Toxicological implications. Food Addit. Contam. 15(Suppl.), 45–51.

Droz, P.O., Wu, M.M., Cumberland, W.G., and Berode, M. 1989. Variability in biological monitoring of solvent exposure. I. Development of a population physiological model. Br J Ind Med. 46, 447-60.

Gearhart, J.M., Mahle, D.A., Greene, R.J., Seckel, C.S., Flemming, C.D., Fisher, J.W., and Clewell, H.J. 1993. Variability of physiologically based pharmacokinetic (PBPK) model parameters and their effects on PBPK model predictions in a risk assessment for perchloroethylene (PCE). Toxicol Lett. 68, 131-44.

Gargas, M.L., Tyler, T.R., Sweeney, L.M., Corley, R.A., Weitz, K.K., Mast, T.J., Paustenbach, D.J., and Hays, S.M. 2000. A toxicokinetic study of inhaled ethylene glycol monomethyl ether (2-ME) and validation of a physiologically based pharmacokinetic model for the pregnant rat and human. Toxicol Appl Pharmacol. 165, 53-62.

Gentry, P.R., Covington, T.R., and Clewell, H.J. 2003. Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. Regul Toxicol Pharmacol. 38, 1-16.

Ghantous, H., and Danielsson, B.R. 1986. Placental transfer and distribution of toluene, xylene and benzene, and their metabolites during gestation in mice. Biol Res Pregnancy Perinatol. 7, 98-105.

Ginsberg, G., Hattis, D., Russ, A., and Sonawane, B. 2004. Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: implications for assessing children's risks from environmental agents. J Toxicol Environ Health 27: 297-329.

Haddad, S., Pelekis, M., and Krishnan, K. 1996. A methodology for solving physiologically based pharmacokinetic models without the use of simulation softwares. Toxicol Lett. 85, 113-26.

International Commission on Radiological Protection (ICRP) 2002. Basic anatomical and physiological data for use in radiological protection: reference values. A report of age- and gender-related differences in the anatomical and physiological characteristics of reference individuals. ICRP Publication 89. Ann ICRP. 32, 5-265.

International Program on Chemical Safety (IPCS). 2001. Guidance Document for the Use of Data in Development of Chemical-Specific Adjustment Factors (CSAFs) for Interspecies Differences and Human Variability in Dose/Concentration–Response Assessment. World Health Organization, Geneva.

Jarabek A.M. 1995. The application of dosimetry models to identify key processes and parameters for default dose-response assessment approaches. Toxicol Lett. 79, 171-84.

Johnsrud, E.K., Koukouritaki, S.B., Divakaran, K., Brunengraber, L.L., Hines, R.N., and McCarver, D.G. 2003. Human hepatic CYP2E1 expression during development. J Pharmacol Exp Ther. 307, 402-7.

Lipscomb, J.C., Teuschler, L.K., Swartout, J., Popken, D., Cox, T., and Kedderis, G.L. 2003. The impact of cytochrome P450 2E1-dependent metabolic variance on a risk-relevant pharmacokinetic outcome in humans. Risk Anal. 23, 1221-38.

Little, B.B. 1999. Pharmacokinetics during pregnancy: evidence-based maternal dose formulation. Obstet Gynecol. 93, 858-68.

Mattison, D.R., Blann, E., and Malek, A. 1991. Physiological alterations during pregnancy: impact on toxicokinetics. Fundam Appl Toxicol. 16, 215-8.

Nong, A., McCarver, D.G., Hines, R.N., and Krishnan, K. 2006. Modeling interchild differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: a case study with toluene. Toxicol Appl. Pharmacol. doi:10.1016/j.taap.2005.12.001

O'Flarherty, E. 1998. PB/PK models. in Handbook of Developmental Neurotoxicology. W. Slikker, L.W. Chang. Eds. Academic Press, San Diego, CA. p. 307-320.

Portier, C.J., and Kaplan, N.L. 1989. Variability of safe dose estimates when using complicated models of the carcinogenic process. A case study: methylene chloride. Fundam Appl Toxicol. 13, 533-44.

Price K., Haddad S., and Krishnan K. 2003a. Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. J Toxicol Environ Health A. 66, 417-33.

Price, P.S., Conolly, R.B., Chaisson, C.F., Gross, E.A., Young, J.S., Mathis, E.T., and Tedder, D.R. 2003b. Modeling interindividual variation in physiological factors used in PBPK models of humans. Crit Rev Toxicol. 33, 469-503.

Syme, M.R., Paxton, J.W., and Keelan, J.A. 2004. Drug transfer and metabolism by the human placenta. Clin Pharmacokinet. 43, 487-514.

Tardif, R., Lapare, S., Charest-Tardif, G., Brodeur, J., and Krishnan, K. 1995. Physiologically-based pharmacokinetic modeling of a mixture of toluene and xylene in humans. Risk Anal. 15, 335-42.

Thomas, R.S., Bigelow, P.L., Keefe, T.J., and Yang R.S. 1996. Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. Am Ind Hyg Assoc J. 57, 23-32.

Vieira, I., Sonnier, M., and Cresteil, T. 1996. Developmental expression of CYP2E1 in the human liver. Hypermethylation control of gene expression during the neonatal period. Eur. J. Biochem. 238, 476–483.

Table 6-1 2001 Canada census population data of Montreal and Nunavik.

Population subgroup	Montreal		Nunavik	
	Individuals	Percent Population	Individuals	Percent Population
Neonate and Toddler (0-4 yrs)	187625	5%	1270	13%
Child and Adolescent (5-19 yrs)	634558	19%	3406	35%
Adult (20-44 yrs)	1287919	38%	3396	35%
Late Adulthood (44-64 yrs)	843205	25%	1120	12%
Elderly (65 to 90 yrs)	442725	13%	260	3%
Pregnant women (14-45 yrs)	30324	1%	172	2%
Total Population	**3426355**	**100%**	**9625**	**100%**

Source: Statistics Canada.

Table 6-2 Physiological data (mean, ranges) used in the present study.

Parameters	Neonate and Toddler	Child and Adolescent	Adult	Late Adulthood	Elderly
Average Age (yrs)	2	10	32	55	75
Body weight (kg)[a]	12 (6.1-19)	37 (17-79)	75 (48.1-119)	78 (51-120)	72.0 (46-110)
Tissue Volumes (L)[a]					
Liver	0.30 (0.20-0.50)	0.80 (0.50-1.5)	1.4 (1.0-2.1)	1.5 (1.1-2.0)	1.4 (1.0-1.9)
Fat	5.1 (2.3-9.3)	11 (2.3-33)	31 (12-63)	33 (14-63)	29.8 (12-54)
Richly perfused tissues	1.9 (1.1-2.6)	4.0 (2.4-6.2)	5.8 (4.5-7.6)	5.9 (4.6-7.5)	5.5 (4.4-6.9)
Poorly perfused tissues			Difference		
Cardiac output (L/hr)a	100 (60-150)	240 (130-420)	420 (310-610)	440 (320-610)	404 (300-560)
Alveolar ventilation (L/hr)[a]	240 (120-350)	480 (320-800)	580 (400-830)	560 (390-800)	483 (350-670)
Tissue Blood Flows (L/hr)[a]					
Liver	19 (11-28)	44 (24-78)	80 (59-120)	83 (61-120)	77.2 (56-110)
Fat	7.6 (3.0-15)	16 (2.8-49)	47 (16-110)	51 (18-110)	45.6 (15-94)
Richly perfused tissues			Difference		
Poorly perfused tissues	11 (5.6-17)	40 (18-89)	92 (67-130)	94 (69-130)	87.9 (64-120)
CYP2E1 liver content (pmol/mg MSP)[b,c]	30 (0.0-86)	57 (23-95)	49 (11-130)	49 (11-130)	48.9 (11-130)

[a] P^3M version 1.2 (Linea Inc, Cape Elizabeth, ME).
[b] Nong et al. 2006.
[c] Lipscomb et al. 2003.

Table 6-3 Physiological data (mean, ranges) for pregnant women used in the present study.

Parameters	Pregnant Women
Average Age (yrs)	30
Body weight (BW kg)[a,b]	81 (40 - 130)
Fraction to BW tissue volumes[a,b]	
Fat	0.27 (0.080 - 0.47)
Liver	0.026 (0.00060 - 0.046)
Richly perfused tissues	0.10 (0.010 - 0.19)
Poorly perfused tissues	Difference
Placenta	0.50 (0.26 - 0.90)
Cardiac output (CO L/hr)[a]	440 (210 - 840)
Alveolar ventilation (L/hr)[a]	440 (210 - 840)
Fraction to CO blood flows[a,b]	
Fat	0.05 (0.010 - 0.10)
Liver	0.05 (0.010 - 0.09)
Richly perfused tissues	Difference
Poorly perfused tissues	0.25 (0.010 - 0.47)
Placenta	0.12 (0.010 - 0.25)
CYP2E1 liver content (pmol/mg MSP)[c]	49 (11 - 130)
Fetal volume (L/hr scaled by $BW^{0.75}$)[a]	3.4 (1.4 - 7.4)
Fetal-Placental blood flow (L/hr)[a]	1.0 (0.22 - 3.7)

[a] Clewell et al. 1999.
[b] ICRP 2002.
[c] Lipscomb et al. 2003.

Table 6-4 Tissue partition coefficients for toluene physiologically based pharmacokinetic (PBPK) model.

Tissue : Air/Blood Partition	Coefficients
Blood : Air[a]	15.6
Liver : Air[a]	83.6
Fat : Air[a]	1020
Richly Perfused Tissues : Air[a]	83.6
Poorly Perfused Tissues : Air[a]	27.7
Placenta : Air[b]	55.0
Fetus : Blood[b]	2.00

[a] Haddad et al. 2001.
[b] Calculated from Ghantous and Danielsson 1986.

Table 6-5 **IVF-PK derived from area under the venous blood concentration versus time curves (AUC) of toluene using Montreal population data.**

Population subgroup	AUC percentiles (mg/L.hr)			IVF-PK	
	5th	50th	95th	95th vs Adult 50th [a]	95th vs Whole Population 50th [b]
Neonate and Toddlers (0-4 yrs)	0.28	0.43	0.60	2.8	2.5
Children and Adolescents (5-19 yrs)	0.24	0.40	0.60	2.8	2.5
Adults (20-44 yrs)	0.14	0.21	0.33	1.5	1.4
Late Adulthood (44-64 yrs)	0.14	0.21	0.32	1.5	1.4
Elderly (65-90 yrs)	0.13	0.19	0.29	1.3	1.2
Pregnant women (14-45 yrs)	0.34	0.50	0.70	3.3	3.0
Whole Population	**0.14**	**0.24**	**0.58**	**2.7**	**2.4**

[a] The **Adult-subpopulation** variability factor was calculated as the ratio of 95th percentile value in each subpopulation over the 50th value for the adult

[b] The **Whole-subpopulation** variability factor was calculated as the ratio of the 95th percentile value in each subpopulation over the 50th percentile value for the whole population.

Table 6-6 IVF-PK derived from area under the venous blood concentration versus time curves (AUC) of toluene using Nunavik region data.

Population subgroup	AUC percentiles (mg/L.hr)			IVF-PK	
	5th	50th	95th	95th vs Adult 50th [a]	95th vs Whole Population 50th [b]
Neonate and Toddlers (0-4 yrs)	0.29	0.43	0.60	2.8	2.2
Children and Adolescents (5-19 yrs)	0.24	0.40	0.61	2.8	2.2
Adults (20-44 yrs)	0.14	0.22	0.33	1.5	1.2
Late Adulthood (44-64 yrs)	0.14	0.22	0.32	1.5	1.2
Elderly (65-90 yrs)	0.13	0.20	0.28	1.3	1.0
Pregnant women (14-45 yrs)	0.34	0.50	0.67	3.1	2.5
Whole Population	**0.15**	**0.27**	**0.57**	**2.7**	**2.1**

[a] The **Adult-subpopulation** variability factor was calculated as the ratio of 95th percentile value in each subpopulation over the 50th value for the adult

[b] The **Whole-subpopulation** variability factor was calculated as the ratio of the 95th percentile value in each subpopulation over the 50th percentile value for the whole population.

Figure Legends

Figure 6-1 Description of the approach for estimating population-specific inter-individual variability factor using demographic data.

Figure 6-2 Geographic representation of the metropolitan Montreal area and Nunavik artic region in the province of Quebec in Canada.

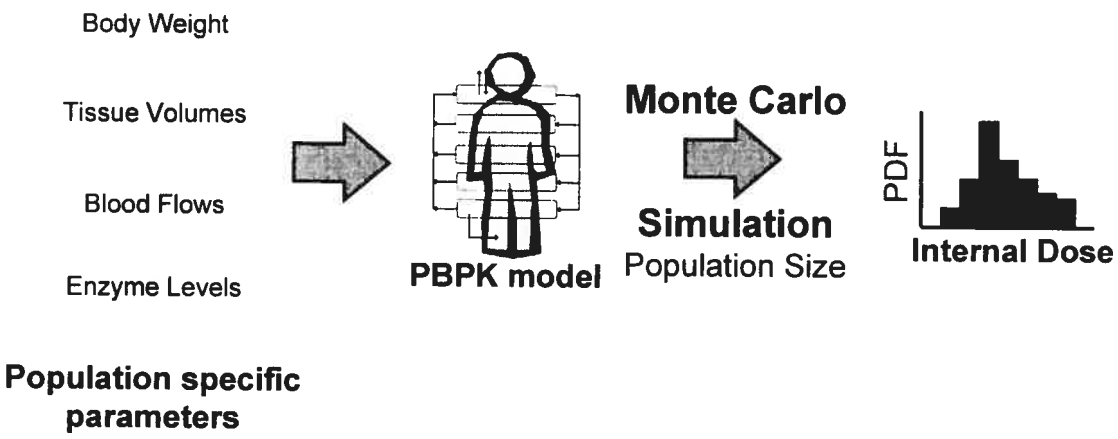
Figure 6-3 Inhalation physiologically based pharmacokinetic (PBPK) model for toluene. Foetal and placental compartment are added to model pregnant women.

Figure 6-4 Probability distributions (PDF) of 24-hr area under the blood toluene concentration curve versus time (AUC) in the population of Montreal metropolitan area. Montreal's population is divided as neonate and toddler (0-4 yrs) 5%, child and adolescent (5-19 yrs) 19%, adult (20-44 yrs) 38%, late adulthood (44-64 yrs) 25%, elderly (65-90 yrs) 13%, and pregnant women (14-45 yrs) 1%.

Figure 6-5 Probability distributions (PDF) of 24-hr area under the blood toluene concentration curve versus time (AUC) in the population of Nunavik region. Nunavik's population is divided as neonate and toddler (0-4 yrs) 13%, child and adolescent (5-19 yrs) 35%, adult (20-44 yrs) 35%, late adulthood (44-64 yrs) 12%, elderly (65-90 yrs) 3%, and pregnant women (14-45 yrs) 2%.

Figure 6-6 Fractional sensitivity of population group specific PBPK model parameters towards the area under the blood toluene concentration curve versus time (AUC). Sensitivity coefficients were calculated as the percent contribution of parameter to the variance of AUC. The most significant parameters by population specific subgroups were body weight (A), fat blood flow (B), liver blood flow (C) and liver CYP2E1 concentration (D).

Figure 6-1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PBPK model
Monte Carlo
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Population Size
PDF
Internal Dose

Figure 6-2

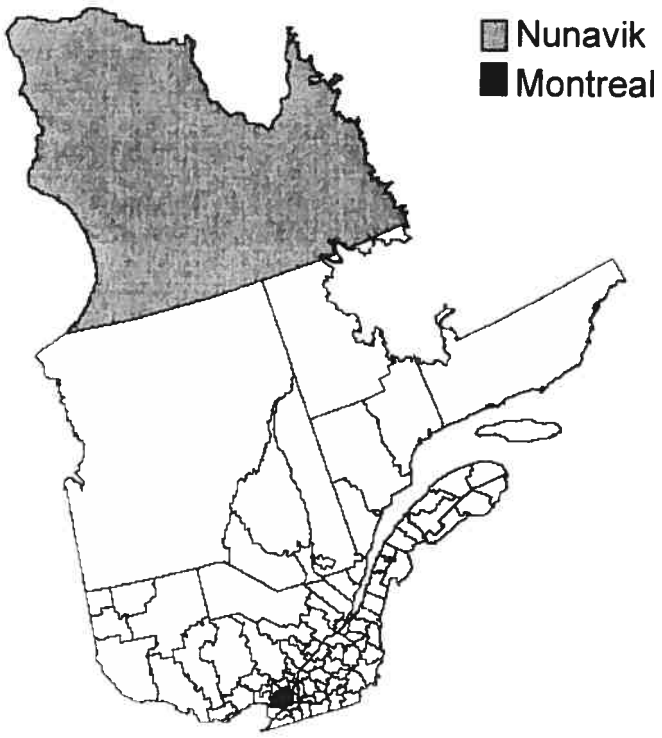

Figure 6-3

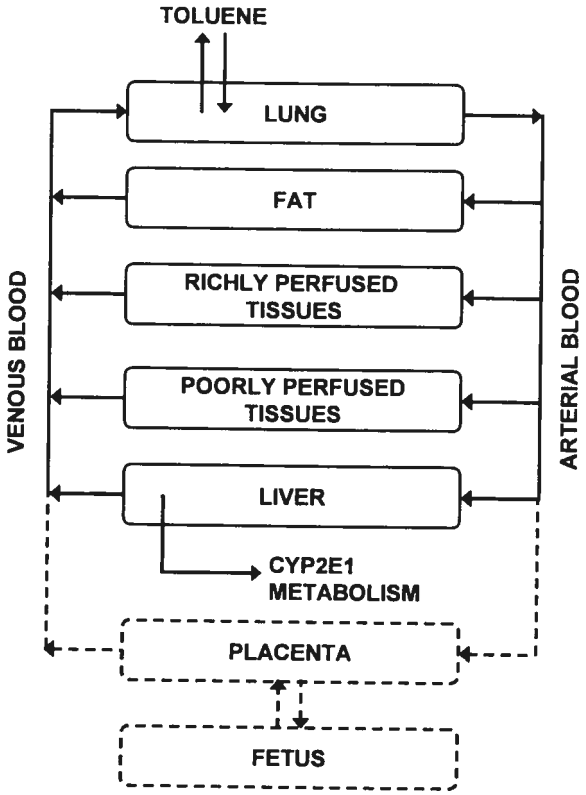
TOLUENE
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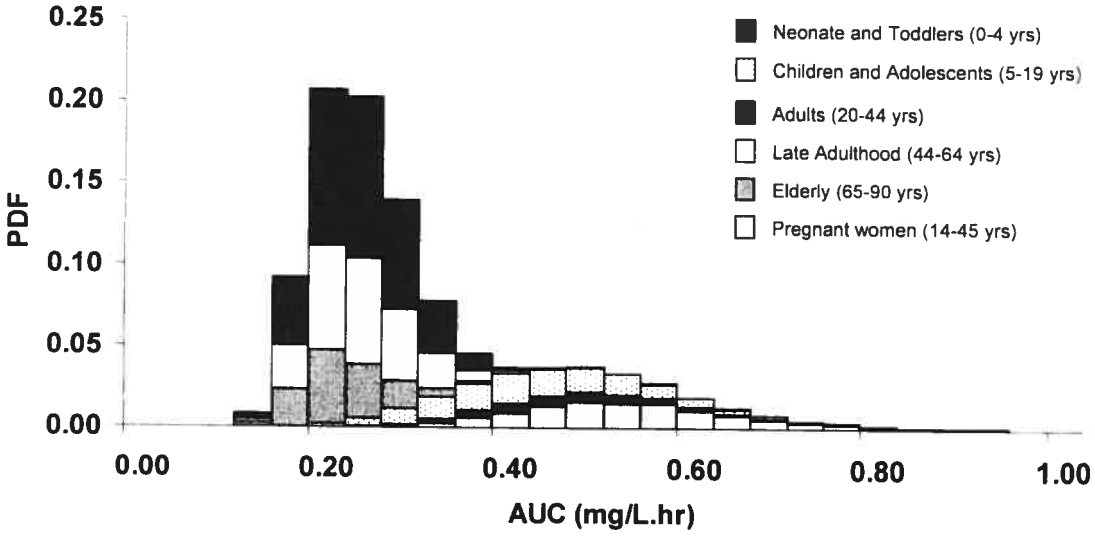
Figure 6-4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Neonate and Toddlers (0-4 yrs)
Children and Adolescents (5-19 yrs)
Adults (20-44 yrs)
Late Adulthood (44-64 yrs)
Elderly (65-90 yrs)
Pregnant women (14-45 yrs)

Figure 6-5

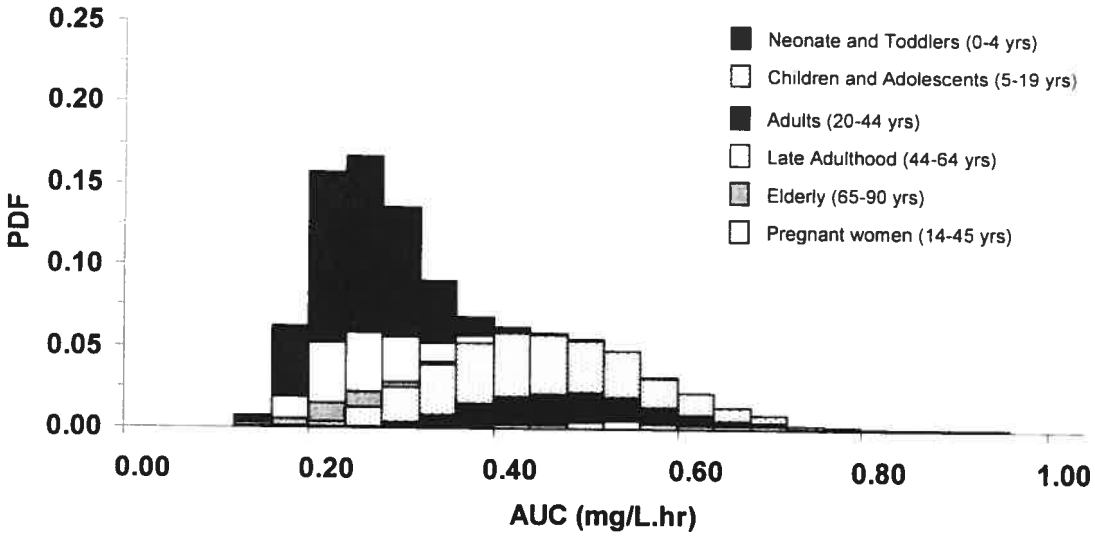
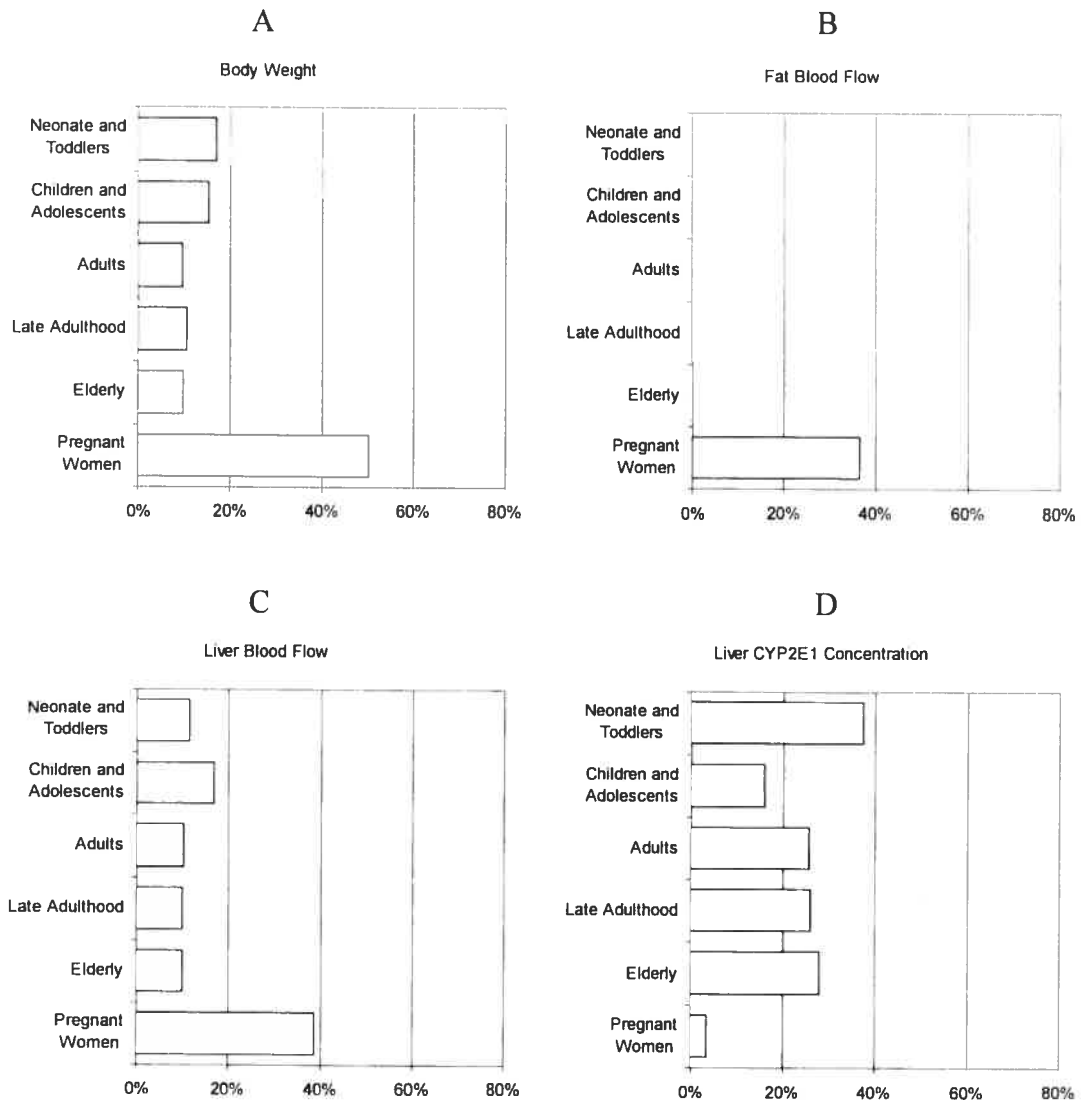

0.25
0.20
0.15
0.10
0.05
0.00
PDF
0.00
0.20
0.40
0.60
0.80
1.00
AUC (mg/L.hr)
Neonate and Toddlers (0-4 yrs)
Children and Adolescents (5-19 yrs)
Adults (20-44 yrs)
Late Adulthood (44-64 yrs)
Elderly (65-90 yrs)
Pregnant women (14-45 yrs)

Figure 6-6


A
Body Weight
Neonate and Toddlers
Children and Adolescents
Adults
Late Adulthood
Elderly
Pregnant Women
0%
20%
40%
60%
80%
B
Fat Blood Flow
Neonate and Toddlers
Children and Adolescents
Adults
Late Adulthood
Elderly
Pregnant Women
0%
20%
40%
60%
80%
C
Liver Blood Flow
Neonate and Toddlers
Children and Adolescents
Adults
Late Adulthood
Elderly
Pregnant Women
0%
20%
40%
60%
80%
D
Liver CYP2E1 Concentration
Neonate and Toddlers
Children and Adolescents
Adults
Late Adulthood
Elderly
Pregnant Women
0%
20%
40%
60%
80%

CHAPITRE VII

7. DISCUSSION GÉNÉRALE

7.1. DISCUSSION

Les articles précédents exposent différentes approches pour caractériser la variabilité interindividuelle affectant la toxicocinétique d'une substance chez une population exposée selon l'étendue des données disponibles. Des données de différentes envergures sur les populations sont exploitées par quatre méthodes selon que ces données soient peu détaillées ou riches en valeurs individuelles, qu'il s'agisse d'un regroupement d'éléments provenant d'une variété d'études sur un groupe de personnes ou que ce soit des statistiques démographiques. Une approche a été développée en fonction de chaque type d'information. Ces approches se traduisent par des calculs par bornes de probabilité avec des doses internes à l'état stationnaire, des simulations PBPK individuelles pour une population, de la modélisation Bayésienne de sous-groupes de population, et de la modélisation Monte Carlo d'une population générale. Les techniques de modélisation PBPK permettent d'appliquer les diverses données de population dans l'analyse de risque à la santé publique aux contaminants environnementaux, tels que les composés organiques volatiles. Même si ces méthodes permettent de caractériser la variabilité interindividuelle toxicocinétique en considérant les différences physiologiques et métaboliques d'une population, elles possèdent néanmoins des contraintes précisées lors de l'application des données. Dans ce dernier chapitre, seront discutées les limites des méthodes de modélisation PBPK de population ainsi que leurs perspectives d'utilisation pour établir des normes d'exposition sécuritaires en santé publique.

7.1.1. Le cadre des approches probabilistes de caractérisation à partir des connaissances disponibles

Le calcul par bornes de probabilité (Probability-bounds) à l'aide d'un algorithme prédisant une concentration sanguine à l'état stationnaire a été utilisé dans le cas où les données disponibles sont pauvres en information populationnelle. Ces données sont souvent des critères statistiques simples comme une moyenne ou des limites (minimum et maximum). En raison de la nature des éléments peu détaillés, un niveau d'incertitude peut s'introduire dans le calcul par bornes de probabilité. De plus, l'interdépendance

entre les déterminants physiologiques peut affecter la confiance sur l'estimation des valeurs limites (Ferson 1996). Toutefois, l'analyse probabiliste permet de caractériser la variabilité interindividuelle toxicocinétique dans une population à partir des évidences scientifiques disponibles alors que la méthode conventionnelle du facteur de 10 (ou 3.2 pour la variabilité interindividuelle TK) utilisée par défaut ignore cette possibilité. D'ailleurs, le calcul par bornes de probabilité avec l'algorithme à l'état stationnaire considère l'aspect mécanistique de la toxicocinétique de la substance contrairement aux approches conventionnelles où on estime une dose de référence en se référant uniquement aux variations des facteurs d'exposition (Swartout *et coll.* 1998, USEPA 1997). En tenant compte des limites introduites par les données, la méthode par bornes de probabilité constitue un calcul scientifique et raisonnable des différences physiologiques et métaboliques dans une population.

D'autre part, une méthode de modélisation PBPK de population a été développée avec des données détaillées sur plusieurs individus. Des mesures de concentration hépatique du cytochrome 2E1 ont été obtenues chez une centaine d'enfants de différents âges (Jonhsrud *et coll.* 2001). À partir de cette collection de mesures, un modèle PBPK a été produit pour chaque enfant. Le modèle physiologique a été adapté selon l'âge et la clairance métabolique spécifique estimée chez ces enfants. Généralement, les valeurs moyennes des déterminants du modèle sont utilisées (Krishnan et Johanson 2005), alors que la population modélisée dans cet exemple correspond à un véritable groupe d'individus. C'est ainsi que la caractérisation de la variabilité interindividuelle par la modélisation individuelle d'une population est beaucoup plus fiable, grâce au nombre de cas étudiés. Par contre, il est rare pour des raisons éthiques d'obtenir un aussi grand nombre de mesures biologiques invasives chez l'humain. En plus, il faut supposer que les différences biologiques observées soient transposables à d'autres populations, ce qui n'est pas évidant en raison du polymorphisme qui existe entre divers groupes ethniques (Kim *et coll.* 1995, Marchand *et coll.* 1999, Nishimoto *et coll.* 2000, Lucas *et coll.* 2001, Bolt *et coll.* 2003). Bien que la caractérisation de la variabilité TK dans une population soit admissible avec ces données riches en information populationnelle, il faut se méfier des hypothèses sur les caractéristiques biologiques d'une population. La méthode

développée pour la série de données individuelles a permis de caractériser la variabilité interindividuelle pour la toxicocinétique du toluène entre l'adulte et l'enfant en se basant sur les différences métaboliques et physiologiques.

Plus particulièrement, la modélisation PBPK Bayésienne a permis de déterminer de nouvelles distributions de paramètres physiologiques et biochimiques d'après les connaissances *à priori* et les valeurs expérimentales existantes. La simulation Bayésienne favorise la combinaison d'informations provenant de plusieurs études pour obtenir des estimations *à posteriori* les plus probables possible. Cette approche nécessite une certaine quantité de connaissances afin d'obtenir des estimations raisonnables. En effet, la simulation Bayésienne implique des données sur plusieurs études et une grande quantité de mesures expérimentales. L'exemple présenté au Chapitre 5 utilise quatre études différentes sur le méthyle éther butyle tertiaire (MTBE) et des données de mesures biologiques sur une dizaine d'individus exposés à deux niveaux de concentration inhalée. L'analyse Bayésienne devient convenable lorsque les connaissances proviennent de plusieurs études. Par contre, si ces données sont limitées, une autre méthode serait plus adéquate, comme le calcul par bornes de probabilité. Dans l'exemple de la caractérisation de la toxicocinétique du MTBE, il a été possible de recueillir suffisamment de données. Développée dans le cadre d'analyse du risque de cancer au dichlorométhane par Jonsson et Johanson (2001), la méthode Bayésienne de caractérisation de la toxicocinétique d'une population devrait être applicable pour raffiner les connaissances de d'autres composés organiques volatils comme le MTBE.

La dernière étude sur la caractérisation de la variabilité interindividuelle introduit les statistiques démographiques d'une population. L'approche consiste à simuler la dose interne d'une population générale en fonction de la répartition des individus par groupe d'âge ou par état physiologique, comme les enfants, les adultes, les femmes enceintes et les personnes âgées. Des données de la population de la région métropolitaine de Montréal et de la région nordique du Nunavik fournies par Statistiques Canada ont été utilisées dans cette étude pour simuler la cinétique de chaque individu, selon la taille de la population. La simulation Monte Carlo du modèle PBPK permet de générer la

distribution des concentrations sanguines en fonction des différences physiologiques au sein d'une population spécifique. Le nombre de simulations individuelles par l'approche Monte Carlo est déterminé en fonction de la taille des sous-groupes démographiques. L'incertitude amenée par le nombre d'itérations qui sont générées pendant la simulation Monte Carlo peut être mesurée en utilisant la méthode Monte Carlo en deux dimensions (USEPA 1999). Dans l'analyse d'incertitude, on simule les distributions des déterminants du modèle PBPK mais aussi les variations probables des propriétés statistiques de ces distributions.

D'autre part, pour chaque population simulée par un modèle PBPK, les distributions de paramètres physiologiques (volumes tissulaires, débits sanguins et poids corporel) et de clairance métabolique ont été quantifiées. Les valeurs statistiques des déterminants physiologiques et métaboliques ont été obtenues à partir des écrits scientifiques, des revues bibliographiques et de sondages sur la santé de la population Nord Américaine. La contrainte majeure de cette analyse d'une sous-population est liée au peu de connaissances disponibles sur chaque sous-groupe de population. Effectivement, la capacité de prédire la dose interne chez un sous-groupe de la population dépend du caractère des distributions de déterminants pharmacocinétiques connues. Par exemple, les simulations Monte Carlo pour les femmes enceintes étaient limitées aux données physiologiques rendues disponibles dans quelques ouvrages de référence comme l'ICRP 2002 et Clewell *et coll.* 2001. Ces ouvrages présentent les valeurs statistiques de plusieurs individus. Cependant, des hypoyhèses sur les caractéristiques métaboliques et biochimiques relatives à la substance étudiée (le toluène) ont dû être faites à cause de connaissances limitées chez la femme enceinte. Pour cette raison, le modèle PBPK chez la femme enceinte est représenté par une structure simple avec un compartiment placenta-foetus élémentaire. En tenant compte de ces contraintes, la méthode développée a généré des distributions de doses internes de la population de Montréal et du Nunavik en se basant sur les connaissances scientifiques disponibles, tel que démontré dans le dernier article.

Plusieurs méthodes de modélisation PBPK de population ont été développées en tenant compte de l'étendue des connaissances disponibles pour caractériser la variabilité interindividuelle de la toxicocinétique d'un composé organique volatil. Éventuellement, ces approches serviront dans le cadre de la détermination d'une dose de référence pour une analyse de risque aux COV.

7.1.2. Les perspectives en santé publique sur la caractérisation de la variabilité interindividuelle des COV par modélisation PBPK de population

Les diverses approches de modélisation PBPK présentées dans cette thèse ont été développées en fonction de la disponibilité des données sur les différences TK interindividuelles qui caractérisent les déterminants physiologiques et métaboliques d'une population. L'objectif ultime de ce projet était de développer une stratégie pour des composés organiques volatils. L'approche choisie dépend de la disponibilité des données concernant les déterminants physiologiques et métaboliques pour une population particulière (Figure 7-1). Pour les situations où il est impossible de déterminer la variabilité interindividuelle toxicocinétique, un facteur de variabilité de 3,2 est utilisé par défaut. Lorsque de nouvelles informations sur les différences toxicocinétiques d'une population deviennent disponibles, un nouveau facteur de variabilité peut être calculé selon diverses approches. L'approche par modélisation PBPK développée dans ce projet est illustrée à la Figure 7-1.

Les quatre études présentées décrivent l'application des approches probabilistes de modélisation PBPK dans l'analyse du risque à la santé pour les composés organiques volatils pour des populations spécifiques. Les analyses de variabilité ont été surtout centrées sur le toluène à cause de la grande disponibilité des données sur ce solvant. Par contre, il est possible de faire le même exercice pour d'autres COV bien documentés, comme l'acétate de vinyle, le chloroforme ou le tricholoroéthylene (Clewell et Andersen 2004, Krishnan et Johanson 2005). Cependant, l'évaluation de la variabilité TK interindividuelle pour différents composés organiques volatils est impossible en l'absence d'un minimum de connaissances. Les techniques Bayésiennes permettent toutefois

d'examiner de nouvelles valeurs de variabilité populationnelle à partir de connaissances à priori et de données expérimentales pour des substances dont la caractérisation de la variabilité de population n'a pas été possible. L'exemple du MTBE a montré que l'on pouvait combiner une variété de données expérimentales avec des données sur divers déterminants physiologiques d'une population. Les nouvelles distributions spécifiques générées pour une substance peuvent ensuite servir à caractériser la variabilité interindividuelle avec une méthode de modélisation PBPK Monte Carlo.

Les exercices de caractérisation de la variabilité interindividuelle par modélisation PBPK permettent de constater que les différences toxicocinétiques interindividuelles peuvent varier d'une population à l'autre. Chacune des méthodes de caractérisation estime un facteur de variabilité interindividuelle spécifique à la population étudiée. Notre étude montre que le facteur de variabilité TK interindividuelle pour des composés organiques volatils lorsqu'uniquement établi pour une population adulte est aussi conservateur que le facteur de 3,2 utilisé par défaut (Jarabek 1995, Barton 2005). Or, la caractérisation de la variation TK interindividuelle entre les adultes et les enfants stipule que les différences métaboliques et physiologiques sont significatives. De telle sorte que l'importance de la variabilité interindividuelle au niveau de la dose interne du toluène entre les adultes et les enfants est égale ou supérieure au facteur de 3,2. Aussi, des facteurs de variabilité TK interindividuelle de plus de 3,2 ont été estimés entre les femmes enceintes et les adultes. Une différence significative au niveau des déterminants toxicocinétiques entre une population adulte et une population sensible explique l'écart obtenu entre les concentrations internes. Une analyse de sensibilité portant sur les déterminants toxicocinétiques de la variation de la dose interne a confirmé l'influence des différences entre populations. Les différences d'élimination par excrétion urinaire ou par voies métaboliques n'ont pas été évaluées. Ces mécanismes biologiques peuvent aussi entraîner une modification du facteur de variabilité interindividuelle en affectant davantage d'autres sous-groupes de population. Les différences physiologiques et métaboliques étudiées entre les sous-groupes de population sont dépendantes de l'âge, de la croissance ou de la grossesse. La modélisation des processus toxicocinétiques selon le développement physiologique de chaque individu d'une population fournit des éléments

importants pour interpréter l'impact des différences biologiques dans une population exposée à des substances volatiles (Clewell *et coll.* 2002, Barton 2005).

Les articles présentés dans cette thèse et qui portent sur la caractérisation de la distribution de la dose interne d'un composé organique volatil présentent une stratégie d'estimation du facteur de variabilité interindividuelle pour une population en considérant l'impact du statut physiologique (ex. grossesse) ou des divers stades de développement physiologiques (ex. enfant, adulte).

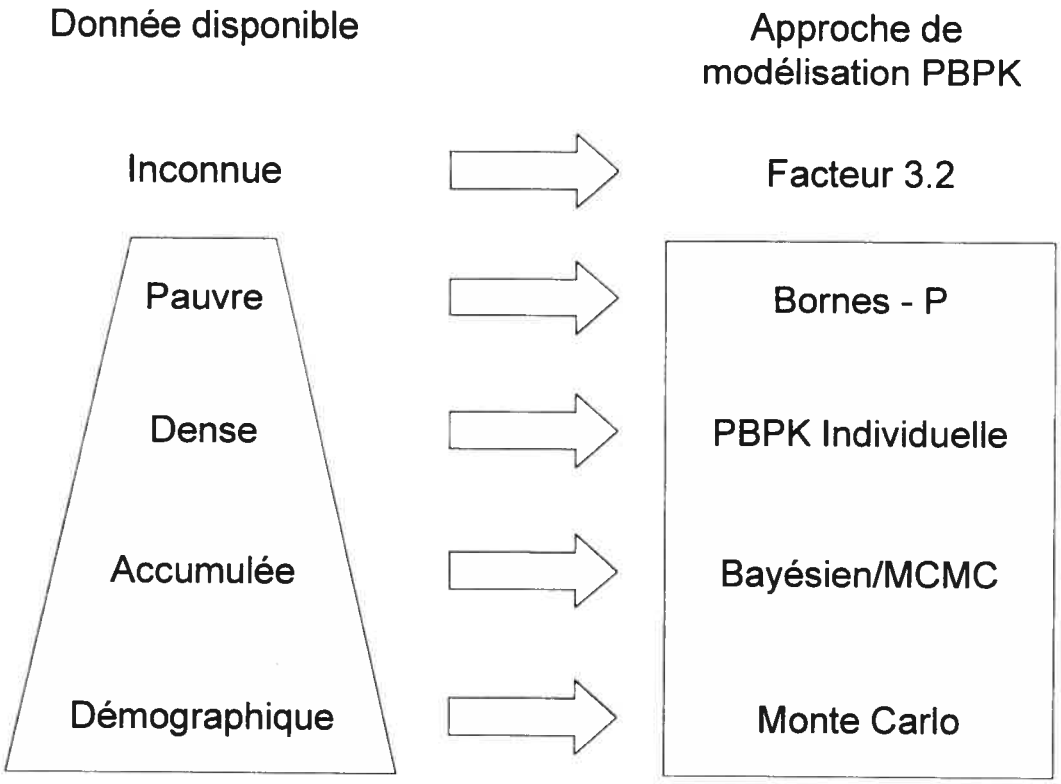


Figure 7-1 Schéma représentatif de la structure d'une analyse par modélisation PBPK pour la caractérisation de la variabilité interindividuelle toxicocinétique des composés organiques volatils.

8. CONCLUSION

En conclusion, les méthodes de modélisation physiologique présentées offrent une stratégie originale permettant de caractériser la variabilité interindividuelle dont on doit tenir compte dans une analyse du risque pour des composés organiques volatils. Selon l'étendue des connaissances toxicocinétiques pour une population et un produit chimique, une approche de modélisation spécifique peut être appliquée. Les approches spécifiques proposées sont : la simulation pharmacocinétique à base physiologique (PBPK) individuelle, l'analyse Bayésienne, la simulation PBPK Monte Carlo, et le recours aux bornes de probabilité. Plusieurs formats de regroupement de données ont été identifiés selon la quantité et le type d'information. Parmi les formats utilisés, on retrouve des valeurs statistiques peu nombreuses, des mesures individuelles d'un groupe riches en information, une série d'études sur plusieurs personnes, des statistiques démographiques urbaines. Malgré la possibilité de prédire la distribution de la dose interne d'une population, l'utilité de ces approches dépend des contraintes associées aux données exploitées. En tenant compte de leurs limites, les méthodes développées offrent la possibilité d'estimer scientifiquement un facteur de variabilité interindividuelle à partir des connaissances disponibles. Les outils développés serviront à l'analyse du risque dans le cadre de l'établissement de niveaux sécuritaires d'exposition aux contaminants environnementaux.

9. BIBLIOGRAPHIE

Agency for Toxic Substances and Disease Registry (ATSDR). 2000. *Toxicological profile for toluene*. U.S. Department Of Health And Human Services, Atlanta, Georgia.

Alcorn, J., McNamara, P.J. 2002a. Ontogeny of hepatic and renal systemic clearance pathways in infants: part I. *Clin Pharmacokinet*. 41(12), 959-98.

Alcorn, J., McNamara, P.J. 2002b. Ontogeny of hepatic and renal systemic clearance pathways in infants: part II. *Clin Pharmacokinet*. 41(13), 1077-94.

Alcorn, J., McNamara, P.J. 2003. Pharmacokinetics in the newborn. *Adv Drug Deliv Rev*. 55(5), 667-86.

Allen, B.C., Covington, T.R., Clewell, H.J. 1996. Investigation of the impact of pharmacokinetic variability and uncertainty on risks predicted with a pharmacokinetic model for chloroform. *Toxicology*. 111, 289-303.

American Conference of Governmental Industrial Hygienists (ACGIH). 2005. *The documentation of the Threshold Limit Values and Biological Exposure Indices*. ACGIH, Cincinnati, Ohio.

Andersen, M.E. 1981. Pharmacokinetics of inhaled gases and vapors. *Neurobehavioral Toxicol and Teratol* 3, 383-389.

Andersen, M.E. 1995. Development of physiologically based pharmacokinetic and physiologically based pharmacodynamic models for applications in toxicology and risk assessment. *Toxicol. Lett.* 79, 35-44.

Andersen, M.E. 2003. Toxicokinetic modeling and its applications in chemical risk assessment. *Toxicol. Lett.* 138, 9-27.

Andersen, M.E., Clewell, H.J., Gargas, M.L., MacNaughton, M.G., Reitz, R.H., Nolan, R.J., McKenna, M.J. 1991. Physiologically based pharmacokinetic modeling with dichloromethane, its metabolite, carbon monoxide, and blood carboxyhemoglobin in rats and humans. *Toxicol. Appl. Pharmacol.* 108, 14-27.

Andersen, M.E., Clewell, H.J., Gargas, M.L., Smith, F.A., Reitz, R.H. 1987. Physiologically-based pharmacokinetics and risk assessment process for methylene chloride. *Toxicol. Appl. Pharmacol.* 87, 185-205.

Andersen, M.E., Dennison, J.E. 2001. Mode of action and tissue dosimetry in current and future risk assessments. *Sci. Tot. Environ.* 274, 3-14.

Andersen, M.E., Gargas, M.L., Jones, R.A., Jenkins, L.J. 1980. Determination of the kinetic constants for metabolism of inhaled toxicants in vivo by gas uptake measurements. *Toxicol. Appl. Pharmacol.* 54, 116.

Arms, A.D., Travis, C.C. 1988. *Reference physiological parameters in pharmacokinetic modeling.* USEPA, Office of Research and Development, National Center for Environmental Assessment, Washington Office, Washington, DC. US EPA/600/6-88/004.

Barton, H.A. 2005. Computational pharmacokinetics during developmental windows of susceptibility. *J. Toxicol. Environ. Health A.* 68(11-12), 889-900.

Basak, S.C., Mills, D., Hawkins, D.M., El-Marsi, H.A. 2002. Prediction of tissue-air partition coefficients: A comparison of structure-based and property-based methods. *SAR QSAR Environ. Res.* 13, 649-665.

Beck, B.D., Mattuck, R.L., Bowers, T.S., Cohen, J.T., O'Flaherty, E. 2001. The development of a stochastic physiologically-based pharmacokinetic model for lead. *Sci Total Environ.* 274(1-3), 15-9.

Beliveau, M., Lipscomb, J., Tardif, R., Krishnan, K. 2005. Quantitative structure-property relationships for interspecies extrapolation of the inhalation pharmacokinetics of organic chemicals. *Chem. Res. Toxicol.* 18,475-85.

Beliveau, M., Tardif, R., Krishnan, K. 2003. Quantitative structure-property relationships for physiologically based pharmacokinetic modeling of volatile organic chemicals in rats. *J. Toxicol. Appl. Pharmacology.* 189, 221-232.

Benignus, V.A., Bushnell, P.J., Boyes, W.K. 1998. A dosimetric analysis of behavioral effects of acute toluene exposure in rats and humans. *Toxicol. Sci.* 43: 186-95.

Benignus, V.A., Bushnell, P.J., Boyes, W.K. 2005. Toward cost-benefit analysis of acute behavioral effects of toluene in humans. *Risk Anal.* 25: 447-56.

Bernillon, P., Bois, F.Y. 2000. Statistical issues in toxicokinetic modeling: a bayesian perspective. *Environ. Health. Perspect.* 108 Suppl 5, 883-93.

Bogdanffy, M.S., Jarabek, A.M. 1995. Understanding mechanisms of inhaled toxicants: implications for replacing default factors with chemical-specific data. *Toxicol Lett.* 82-83, 919-32.

Bois, F.Y. 2000. Statistical analysis of Clewell et al. PBPK model of trichloroethylene kinetics. *Environ. Health Perspect.* 108 Suppl 2, 307-16.

Bois, F.Y., Jackson, E.T., Pekari, K., Smith, M.T. 1996. Population toxicokinetics of benzene. *Environ. Health. Perspect.* 104 Suppl 6:1405-11.

Bolt, H.M., Roos, P.H., Their, R. 2003. The cytochrome P-450 isoenzyme CYP2E1 in the biological processing of industrial chemicals: consequences for occupational and environmental medicine. *Int Arch Occup Environ Health.* 76(3), 174-85.

Borghoff, S.J., Murphy, J.E., Medinsky, M.A. 1996. Development of physiologically based pharmacokinetic model for methyl tertiary-butyl ether and tertiary-butanol in male Fisher-344 rats. *Fundam. Appl. Toxicol.* 30, 264-275.

Branum, A.M., Collman, G.W., Correa, A., et al. 2003. The National Children's Study of environmental effects on child health and development. *Environ. Health Perspect.* 111(4), 642-6.

Brown, R.P., Delp, M.D., Lindstedt, S.L., Rhomberg, L.R., Beliles, R.P. 1997. Physiological parameter values for physiologically based pharmacokinetic models. *Toxicol. Ind. Health.* 13, 407-484.

Burmaster, D.E., Anderson, P.D. 1994. Principles of good practice for the use of Monte Carlo techniques in human health and ecological risk assessments. *Risk Anal.* 14(4), 477-81.

Burmaster, D.E., Llyod, K.J., Thompson, K.M. 1995. The need for new methods to backcalculate soil cleanup targets in interval and probabilistic cancer risk assessments *Hum. Ecol. Risk Assessment.* 1, 89-100.

Byczkowski, J.Z., Gearhart, J.M., Fisher, J.W. 1994. "Occupational" exposure of infants to toxic chemicals via breast milk. *Nutrition.* 10(1), 43-8.

Cahalan, M.K., Johnson, B.H., Edger, E.I. 1981. Relationship of concentrations of halothane and enflurane to their metabolism and elimination in man. *Anesthesiology.* 54, 3-8.

Caster, W.O., Poncelet, J., Simon, A.B., Armstrong, W.B. 1956. Tissue weights of the rat. I. Normal values determined by dissection and chemical methods. *Proc. Soc. Exp. Biol. Med.* 91, 122-126.

Clewell, H.J., Andersen, M.E. 1987. Dose species and route extrapolations using physiologically-based pharmacokinetic modeling. *Drinking Water and Health.* 8, 159-184.

Clewell, H.J., Andersen, M.E. 1994. Physiologically-based pharmacokinetic modeling and bioactivation of xenobiotics. *Toxicol. Ind. Health.* 10, 1-24.

Clewell, H.J., Andersen, M.E. 1996. Use of physiologically based pharmacokinetic modeling to investigate individual versus population risk. *Toxicology.* 111, 315-29.

Clewell, H.J., Andersen, M.E. 2004. Applying mode-of-action and pharmacokinetic considerations in contemporary cancer risk assessments: an example with trichloroethylene. *Crit Rev Toxicol.* 34(5), 385-445.

Clewell, H.J., Andersen, M.E., Barton, H.A. 2002. A consistent approach for the application of pharmacokinetic modeling in cancer and noncancer risk assessment. *Environ. Health Perspect.* 110, 85-93.

Clewell, H.J., Gearhart, J.M., Gentry, P.R., Covington, T.R., VanLandingham, C.B., Crump, K.S., Shipp, A.M. 1999. Evaluation of the uncertainty in an oral reference dose for methylmercury due to interindividual variability in pharmacokinetics. *Risk Anal.* 19, 547-58.

Clewell, H.J., Gentry, P.R., Covington, T.R., Sarangapani, R., Teeguarden, J.G. 2004. Evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Toxicol. Sci.* 79,381-93.

Clewell, H.J., Gentry, P.R., Gearhart, J.M., Allen, B.C., Andersen, M.E. 2001. Comparison of cancer risk estimates for vinyl chloride using animal and human data with a PBPK model. *Sci Total Environ.* 274(1-3), 37-66.

Clewell, H.J., Teeguarden, J.G., McDonald, T., Sarangapani, R., Lawrence, G.S., Covington, T.R., Gentry, R., Shipp, A. 2002. Review and evaluation of the potential impact of age- and gender-specific pharmacokinetic differences on tissue dosimetry. *Crit Rev Toxicol* 32, 329-389.

Corley, R.A., Mast, T.J., Carney, E.W., Rogers, J.M., Daston, G.P. 2003. Evaluation of physiologically based models of pregnancy and lactation for their application in children's health risk assessments. *Crit Rev Toxicol.* 33, 137-211.

Cox, L.A. 1996. Reassessing benzene risks using internal doses and Monte-Carlo uncertainty analysis. *Environ. Health Perspect.* 104 Suppl 6, 1413-29.

Cresteil, T. 1998. Onset of xenobiotic metabolism in children: Toxicological implications. *Food Addit. Contam.* 15(Suppl.), 45–51.

Cronin, W.J., Oswald, E.J., Shelley, M.L., Fisher, J.W., Flemming, C.D. 1995. A trichloroethylene risk assessment using a Monte Carlo analysis of parameter uncertainty in conjunction with physiologically-based pharmacokinetic modeling. *Risk Anal.* 15(5), 555-65.

Cusack, B.J. 2004. Pharmacokinetics in older persons. *Am J Geriatr Pharmacother.* 2(4), 274-302.

Dallas, C.E., Muralidhara, S., Chen, X.M., Ramanthan, R., Varkonyl, P., Gallo, J M., Bruckner, J.V. 1994. Use of a physiologically based model to predict systemic uptake and respiratory elimination of perchloroethylene. *Toxicol. Appl. Pharmacol.* 128, 60-68

Daston, G., Faustman, E., Ginsberg, G., Fenner-Crisp, P., Olin, S., Sonawane, B., Bruckner, J., Breslin, W., McLaughlin, T.J. 2004. A framework for assessing risks to

children from exposure to environmental agents. *Environ. Health Perspect.* 112(2), 238-56.

Davies, B., Morris, T. 1993. Physiological parameters in laboratory animals and humans. *Pharm. Res.* 10, 1093-1095

de Zwart, L.L., Haenen, H.E., Versantvoort, C.H., Wolterink, G., van Engelen, J.G., Sips, A.J. 2004. Role of biokinetics in risk assessment of drugs and chemicals in children. *Regul. Toxicol. Pharmacol.* 39(3), 282-309.

Dedrick, R.L., Forrester, D.D., Ho, D.H.W. 1972. In vitro-in vivo correlation of drug metabolism: Deamination of 1-β-D-arabinosyl cytosine. *Biochem. Pharmacol.* 21, 1-16.

Dedrick, R.L., Zaharko, D.S., Lutz, R.J. 1973. Transport and binding of methotrexate in vivo. *J. Pharm. Sci.* 62, 882-890.

DeJongh, J., Blaauboer, B.J. 1997. Evaluation of in vitro-based simulations of toluene uptake and metabolism in rats. *Toxicol. in Vitro.* 11, 485-489.

Delic, J.I., Lilly, P.D., MacDonald, A.J., Loizou, G.D. 2000. The utility of PBPK in the safety assessment of chloroform and carbon tetrachloride. *Regul. Toxicol. Pharmacol.* 32, 144-55.

Delp, M.D., Manning, R.O., Bruckner, J.V., Armstrong, R.B. 1991. Distribution of cardiac output during diurnal changes in activity in rats. *Am. J. Physiol.* 261, H1487-1493.

Dennison, J.E., Andersen, M.E., Yang, R.S. 2005. Pitfalls and related improvements of in vivo gas uptake pharmacokinetic experimental systems. *Inhal. Toxicol.* 17,539-48.

Dodds, M.G., Vicini, P. 2004. Assessing convergence of Markov chain Monte Carlo simulations in hierarchical Bayesian models for population pharmacokinetics. *Ann. Biomed. Eng.* 32, 1300-13.

Dorne, J.L. 2004. Impact of inter-individual differences in drug metabolism and pharmacokinetics on safety evaluation. *Fundam Clin Pharmacol.* 18, 609-20.

Dorne, J.L., Renwick, A.G. 2005. The refinement of uncertainty/safety factors in risk assessment by the incorporation of data on toxicokinetic variability in humans. *Toxicol. Sci.* 86, 20-26.

Dorne, J.L., Walton, K., Renwick, A.G. 2003. Human variability in the renal elimination of foreign compounds and renal excretion-related uncertainty factors for risk assessment. *Food Chem Toxicol.* 42, 275-298.

Dorne, J.L., Walton, K., Renwick, A.G. 2004. Human variability for metabolic pathways with limited data (CYP2A6, CYP2C9, CYP2E1, ADH, esterases, glycine and sulphate conjugation). *Food Chem Toxicol.* 42, 397-421.

Dorne, J.L., Walton, K., Renwick, A.G. 2005. Human variability in xenobiotic metabolism and pathway-related uncertainty factors for chemical risk assessment: a review. *Food Chem Toxicol.* 43, 203-16.

Dourson, M.L., Charnley G., Scheuplein R. 2002. Differential sensitivity of children and adults to chemical toxicity. II. Risk and regulation. *Regul. Toxicol. Pharmacol.* 35, 448-67.

Dourson, M.L., Felter, S.P., Robinson, D. 1996. Evolution of science-based uncertainty factors in noncancer risk assessment. *Regul. Toxicol. Pharmacol.* 24:108-20.

Droz, P.O., Wu, M.M., Cumberland, W.G. 1989b. Variability in biological monitoring of organic solvent exposure. II. Application of a population physiological model. *Br J Ind Med.* 46(8), 547-58.

Droz, P.O., Wu, M.M., Cumberland, W.G., Berode, M. 1989a. Variability in biological monitoring of solvent exposure. I. Development of a population physiological model. *Br J Ind Med.* 46, 447-60.

D'Souza, R.W., Francis, W.R., Andersen, M.W. 1988. Physiological model for tissue glutathione depletion and increased resynthesis after ethylene dichloride exposures. *J. Pharmacol. Exp. Ther.* 245,563-568.

Easterling, M.R., Evans, M.V., Kenyon, E.M. 2000. Comparative analysis of software for physiologically based pharmacokinetic modeling: simulation, optimization, and sensitivity analysis. *Toxicol. Meth.* 10, 203-229.

El-Masri, H.A., Bell, D.A., Portier, C.J. 1999. Effects of glutathione transferase theta polymorphism on the risk estimates of dichloromethane to humans. *Toxicol Appl Pharmacol.* 158(3), 221-30.

Faustman, E.M., Silbernagel, S.M., Fenske, R.A., Burbacher, T.M., Ponce, R.A. 2000. Mechanisms underlying Children's susceptibility to environmental toxicants. *Environ. Health Perspect.* 108 Suppl 1, 13-21.

Ferson, S, Root, W.T., Kuhn, R. 1999. RAMAS Risk Calc: Risk Assessment with Uncertain Numbers. *Applied Biomathematics.* Setauket, New York.

Ferson, S. 1996. What Monte Carlo Methods cannot do. *Hum. Ecol. Risk. Assess.* 2, 990-1007.

Filser, J.G., Bolt, H.M. 1981. Inhalation pharmacokinetics based on gas uptake studies. I. Improvement of kinetic models. *Arch. Toxicol.* 47, 279-292.

Filser, J.G., Csanady, G.A., Kreuzer, P.E., Kessler, W. 1995. Toxicokinetic models for volatile industrial chemicals and reactive metabolites. *Toxicol. Lett.* 82/83, 357-366.

Finley, B.L., Connor, K.T., Scott, P.K. 2003. The use of toxic equivalency factor distributions in probabilistic risk assessments for dioxins, furans, and PCBs. *J. Toxicol. Environ. Health. A.* 66(6), 533-50.

Fiserova-Bergerova, V. 1983. *Modeling of inhalation exopsure to vapors: uptake, distribution and elimination.* Vol. 1. CRC Press Inc., Boca Raton, FL, pp.73-100.

Fiserova-Bergerova, V. 1995. Extrapolation of physiological parameters for physiologically based simulation models. *Toxicol. Lett.* 79, 77-86.

Fiserova-Bergerova, V., Diaz, M.L. 1986. Determination and prediction of tissue-gas partition coefficients. *Int. Arch. Occup. Environ. Health.* 58, 75-87.

Fisher, J., Mahle, D., Bankston, L., Greene, R., Gearhart, J. 1997. Lactational transfer of volatile chemicals in breast milk. *Am. Ind. Hyg. Assoc. J.* 58(6), 425-31.

Foo, S.C., Jeyaratnam, J., D. Koh, D. 1990. Chronic neurobehavioral effects of toluene. *Br. J. Ind. Med.* 47,480-484.

Gargas, M.L. Andersen, M.E., Clewell, H.J. 1986. A physiologically-based simulation approach for determining metabolic rate constants from gas uptake data. *Toxicol. Appl. Pharmacol.* 86, 341-352.

Gargas, M.L. Burgess, R.J, Voisard, D.E, Cason, G.H., Andersen, M.E. 1989. Partition coefficients of low molecular weight volatile chemicals in various liquids and tissues. *Toxicol. Appl. Pharmacol.* 98, 87-99.

Gargas, M.L. 1990. An exhaled breath chamber system for assessing rates of metabolism and rates of gastrointestinal absorption with volatile chemicals. *J. Am. Coll. Toxicol.* 9, 447-453.

Gargas, M.L., Andersen, M.E. 1988. Physiologically based approaches for examining the pharmacokinetics of inhaled vapors. In: Gardner DE, Crapo JD, Massaro EJ, editors. *Toxicology of the lung*. New York: Raven Press. p 449-476.

Gargas, M.L., Andersen, M.E. 1989. Determinating the kinetic constants of chlorinated ethane metabolism in the rat from rates of exhalation. *Toxicol. Appl. Pharmacol.* 99, 344-353.

Gargas, M.L., Clewell, H.J., Andersen, M.E. 1990. Gas uptake inhalation techniques and the rates of metabolism of chloromethanes, chloroethanes and chloroethylenes in the rat. *Inhal. Toxicol.* 2, 319.

Gargas, M.L., Tyler, T.R., Sweeney, L.M., Corley, R.A., Weitz, K.K., Mast, T.J., Paustenbach, D.J., Hays, S.M. 2000. A toxicokinetic study of inhaled ethylene glycol monomethyl ether (2-ME) and validation of a physiologically based pharmacokinetic model for the pregnant rat and human. *Toxicol Appl Pharmacol.* 165, 53-62.

Gearhart, J.M., Mahle, D.A., Greene, R.J., Seckel, C.S., Flemming, C.D., Fisher, J.W., Clewell, H.J. 1993. Variability of physiologically based pharmacokinetic (PBPK) model parameters and their effects on PBPK model predictions in a risk assessment for perchloroethylene (PCE). *Toxicol Lett.* 68, 131-44.

Gehring, P.J., Watanabe, P.G., Park, C.N. 1978. Resolution of dose-response toxicity data for chemicals requiring metabolic activation: example--vinyl chloride. *Toxicol Appl Pharmacol.* 44, 581-91.

Gelman, A., Rubin, D.B. 1996. Markov chain Monte Carlo methods in biostatistics. Stat Methods Med Res. 5(4), 339-55.

Gentry, P.R., Covington, T.R., Clewell, H.J. 2003. Evaluation of the potential impact of pharmacokinetic differences on tissue dosimetry in offspring during pregnancy and lactation. *Regul. Toxicol. Pharmacol.* 38, 1-16.

Gentry, P.R., Hack, C.E., Haber, L.., Maier, A., Clewell, H.J. 2002. An approach for the quantitative consideration of genetic polymorphism data in chemical risk assessment: examples with warfarin and parathion. *Toxicol. Sci.* 70(1), 120-39.

Ghantous, H., Danielsson, B.R. 1986. Placental transfer and distribution of toluene, xylene and benzene, and their metabolites during gestation in mice. *Biol Res Pregnancy Perinatol.* 7, 98-105.

Ginsberg, G., Hattis, D., Russ, A., Sonawane, B. 2004. Physiologically based pharmacokinetic (PBPK) modeling of caffeine and theophylline in neonates and adults: implications for assessing children's risks from environmental agents. *J Toxicol Environ Health* 27: 297-329.

Ginsberg, G., Hattis, D., Sonawane, B. 2004a. Incorporating pharmacokinetic differences between children and adults in assessing children's risks to environmental toxicants. *Toxicol. Appl. Pharmacol.* 198(2), 164-83.

Ginsberg, G., Hattis, D., Sonawane, B., Russ, A., Banati, P., Kozlak, M., Smolenski, S., Goble, R. 2002. Evaluation of child/adult pharmacokinetic differences from a database derived from the therapeutic drug literature. *Toxicol. Sci.* 66, 185-200.

Ginsberg, G., Slikker, W. Jr., Bruckner, J., Sonawane, B. 2004b. Incorporating children's toxicokinetics into a risk framework. *Environ. Health. Perspect.* 112(2), 272-83.

Guengerich F.P., Kim D.H., Iwasaki M. 1991. Role of human cytochrome P-450 IIE1 in the oxidation of many low molecular weight cancer suspects. *Chem. Res. Toxicol.* 4, 168-79.

Haddad, S., Pelekis, M., Krishnan, K. 1996. A methodology for solving physiologically based pharmacokinetic models without the use of simulation softwares. *Toxicol. Lett.* 85, 113-26.

Haddad, S., Restieri, C., Krishnan, K. 2001. Characterization of age-related changes in body weight and organ weights from birth to adolescence in humans. *J. Toxicol. Environ. Health A.* 64(6), 453-64.

Haddad, S., Tardif, R., Viau, C., Krishnan K. 1999. A modeling approach to account for pharmacokinetic interactions in the calculation of biological hazard index for chemical mixtures. *Toxicol. Lett.* 108, 303-308.

Hamelin, G., Charest-Tardif, G., Truchon, G., Tardif, R. 2005. Physiologically based modeling of n-hexane kinetics in humans following inhalation exposure at rest and under physical exertion: impact on free 2,5-hexanedione in urine and on n-hexane in alveolar air. *J. Occup. Environ. Hyg.* 2(2), 86-97.

Hattis, D., L. Erdreich, M. Ballew. 1987. *Human Variability in Susceptibility to Toxic Chemicals: a Preliminary Analysis of Pharmacokinetic Data from Normal Volunteers.* U.S. Environmental Protection Agency, Washington, D.C., EPA/600/J-87/320.

Hines, R.N., McCarver, D.G. 2002. The ontogeny of human drug-metabolizing enzymes: phase I oxidative enzymes. *J. Pharmacol. Exp. Ther.* 300(2),355-60.

International Commission on Radiological Protection (ICRP). 1975. *Report of the Task Group on Reference Man : a report / prepared by a task group of Committee 2 of the International Commission on Radiological Protection.* Oxford; Pergamon Press, Toronto.

International Commission on Radiological Protection (ICRP). 2002. Basic anatomical and physiological data for use in radiological protection: reference values. A report of age- and gender-related differences in the anatomical and physiological characteristics of reference individuals. ICRP Publication 89. *Ann ICRP.* 32, 5-265.

International Life Sciences Institute (ILSI), Risk Science Institute. 1994. *Physiological Parameter Values for PBPK Models.* ILSI, Washington, DC.

International Program on Chemical Safety (IPCS). 2001. *Guidance Document for the Use of Data in Development of Chemical-Specific Adjustment Factors (CSAFs) for Interspecies Differences and Human Variability in Dose/Concentration–Response Assessment*. World Health Organization, Geneva.

International Program on Chemical Safety (IPCS). 2005. *Chemical-specific adjustment factors for interspecies differences and human variability: guidance document for use of data in dose/concentration–response assessment*. WHO Document Production Services, Geneva, Switzerland.

Isukapalli, S.S., Roy, A., Georgopoulos, P.G. 1998. Stochastic response surface methods (SRSMs) for uncertainty propagation: application to environmental and biological systems. *Risk Anal.* 18(3), 351-63.

Jarabek, A.M. 1995. The application of dosimetry models to identify key processes and parameters for default dose-response assessment approaches. *Toxicol. Lett.* 79, 171-84.

Johanson, G, Dynesius, B. 1988. Liquid: air partition coefficients for six commonly used glycol ethers. *Br. J. Ind. Med.* 45, 561-564.

Johanson, G. 1991. Modelling of respiratory exchange of polar solvents, *Ann. Occup. Hyg.*, 35, 323-339.

Johanson, G., Filser, J.G. 1992. Experimental data from closed chamber gas uptake studies in rodents suggest lower uptake rate of chemical than calculated from literature values on alveolar ventilation. *Arch. Toxicol.* 66, 291-295.

Johanson, G., Naslund, P.H. 1988. Spreadsheet programming: A new approach in physiologically based modeling of solvent toxicokinetics. *Toxicol. Lett.* 41, 115-127.

Johanson, G., Nihlen, A., Lof, A. 1995. Toxicokinetics and acute effects of MTBE and ETBE in male volunteers. *Toxicol. Lett.* 82-83:713-8.

Johnsrud, E.K., Koukouritaki, S.B., Divakaran, K., Brunengraber, L.L., Hines, R.N., McCarver, D.G. 2003. Human hepatic CYP2E1 expression during development. *J. Pharmacol. Exp. Ther.* 307, 402-7.

Jonsson, F., Bois, F.Y., Johanson, G. 2001a. Physiologically based pharmacokinetic modeling of inhalation exposure of humans to dichloromethane during moderate to heavy exercise. *Toxicol. Sci.* 59, 209-18.

Jonsson, F., Bois, F.Y., Johanson, G. 2001b. Assessing the reliability of PBPK models using data from methyl chloride-exposed, non-conjugating human subjects. *Arch. Toxicol.* 75, 189-199.

Jonsson, F., Johanson, G. 2001. A Bayesian analysis of the influence of GSTT1 polymorphism on the cancer risk estimate for dichloromethane. *Toxicol. Appl. Pharmacol.* 174, 99–112.

Jonsson, F., Johanson, G. 2001. Bayesian estimation of variability in adipose tissue blood flow in man by physiologically based pharmacokinetic modeling of inhalation exposure to toluene. *Toxicology.* 157(3), 177-93.

Jonsson, F., Johanson, G. 2002. Physiologically based modeling of the inhalation kinetics of styrene in humans using a bayesian population approach. *Toxicol. Appl. Pharmacol.* 179, 35-49.

Jonsson, F., Johanson, G. 2003. The Bayesian population approach to physiological toxicokinetic–toxicodynamic models—an example using the MCSim software. *Toxicol. Let.* 138, 143-150.

Kao, S.C., Bell, E.F., Brown, B.P., Smith, W.L. 1996. Duplex Doppler sonography of changes in portal vein flow in healthy term newborn infants after feeding. *J Ultrasound Med.* 15, 121-5.

Kass, R.E., Wasserman, L. 1996. The selection of prior distribution by formal rules. *J. Am. Stat. Assoc.* 91, 1343-1370.

Kedderis, G.L., Lipscomb, J.C. 2001. Application of in vitro biotransformation data and pharmacokinetic modeling to risk assessment. *Toxicol Ind Health.* 17, 315-21.

Kim, R.B., O'Shea, D. 1995. Interindividual variability of chlorzoxazone 6-hydroxylation in men and women and its relationship to CYP2E1 genetic polymorphisms. *Clin Pharmacol Ther.* 57(6), 645-55.

Koukouritaki, S.B., Manro J.R., Marsh S.A., Stevens J.C., Rettie A.E., McCarver D.G., Hines R.N. 2004. Developmental expression of human hepatic CYP2C9 and CYP2C19. *J. Pharmacol. Exp. Ther.* 308, 965-74.

Krishnan, K., Andersen, M.E. 2001. Physiologically based pharmacokinetic modeling in toxicology. In *Principles and methods of toxicology* (A.W. Hayes, Ed.), Taylor & Francis, Philadelphia, PA, pp. 193-241.

Krishnan, K., Gargas, M.L., Fennell, T.R., Andersen, M E. 1992. A physiologically-based description of ethylene oxide dosimetry in the rat. *Toxicol. Ind. Health.* 8, 121-140.

Krishnan, K., Johanson, G. 2005. Physiologically-based pharmacokinetic and toxicokinetic models in cancer risk assessment. *J. Environ. Sci. Health.* 23, 31-53.

Krishnan, K., Nihlen, A., Ernstgard, L., Johanson, G. 2005. Simulation of the inhalation pharmacokinetics of methyl tert-butyl ether in humans. In: J.G. Anderson and M. Katzper (Eds.), *Proceedings of the 2005 International Health Sciences Simulation Conference* (ISBN 1-56555-289-X). The Society for Computer Simulation International, San Diego, pp 105-110.

Leung, H.W., Paustenbach, D.J. 1995. Physiologically based pharmacokinetic and pharmacodynamic modeling in health risk assessment and characterization of hazardous substances. *Toxicol. Lett.* 79, 55-65.

Licata, A.C., Dekant, W., Smith, C.E., Borghoff, S.J. 2001. A physiologically based pharmacokinetic model for methyl tert-butyl ether in humans: implementing sensitivity and variability analyses. *Toxicol. Sci.* 62, 191-204.

Lipscomb J.C., Barton H.A., Tornero-Velez R., Evans M.V., Alcasey S., Snawder J.E., Laskey J. 2004. The metabolic rate constants and specific activity of human and rat hepatic cytochrome P-450 2E1 toward toluene and chloroform. *J. Toxicol. Environ. Health A.* 67, 537-53.

Lipscomb, J.C., Fisher, J.W., Confer, P.D., Byczkowski, J.Z. 1998. In vitro to in vivo extrapolation for trichloroethylene metabolism in humans. *Toxicol. Appl. Pharmacol.* 152, 376-387.

Lipscomb, J.C., Kedderis, G.L. 2002. Incorporating human interindividual biotransformation variance in health risk assessment. *Sci Total Environ.* 288(1-2), 13-21.

Lipscomb, J.C., Teuschler, L.K., Swartout, J., Popken, D., Cox, T., Kedderis, G.L. 2003. The impact of cytochrome P450 2E1-dependent metabolic variance on a risk-relevant pharmacokinetic outcome in humans. *Risk Anal.* 23, 1221-38.

Little, B.B. 1999. Pharmacokinetics during pregnancy: evidence-based maternal dose formulation. *Obstet Gynecol.* 93, 858-68.

Lof, A., Johanson, G. 1998. Toxicokinetics of organic solvents: a review of modifying factors. *Crit Rev Toxicol.* 28(6), 571-650.

Lof, A., Wigaeus Hjelm, E., Colmsjo, A., Lundmark, B.O., Norstrom, A., Sato A. 1993. Toxicokinetics of toluene and urinary excretion of hippuric acid after human exposure to 2H8-toluene. *Br J Ind Med.* 50, 55-9.

Lucas, D., Ferrara, R., Gonzales, E., Albores, A., Manno, M., Berthou, F. 2001. Cytochrome CYP2E1 phenotyping and genotyping in the evaluation of health risks from exposure to polluted environments. *Toxicol Lett.* 124(1-3), 71-81.

Luecke, R.H., Wosilait, W.D., Pearce, B.A., Young, J.F. 1997. A computer model and program for xenobiotic disposition during pregnancy. *Comput. Methods Programs Biomed.* 53(3), 201-24.

MacDonald, A.J., Rostami-Hodjegan, A., Tucker, G.T., Linkens, D.A. 2002. Analysis of solvent central nervous system toxicity and ethanol interactions using a human population physiologically based kinetic and dynamic model. *Regul. Toxicol. Pharmacol.* 35(2 Pt 1), 165-76.

Maddalena, R.L., McKone, T.E., Sohn, M.D. 2004. Standardized approach for developing probabilistic exposure factor distributions. *Risk Anal.* 24(5), 1185-99.

Marchand, L.L., Wilkinson, G.R., Wilkens, L.R. 1999. Genetic and dietary predictors of CYP2E1 activity: a phenotyping study in Hawaii Japanese using chlorzoxazone. *Cancer Epidemiol. Biomarkers Prev.* 8(6), 495-500.

Mattison, D.R., Blann, E., Malek, A. 1991. Physiological alterations during pregnancy: impact on toxicokinetics. *Fundam Appl Toxicol.* 16, 215-8.

Mauderly, J.L., Measurement of respiration and respiratory responses during inhalation exposures. *J. Am. Coll. Toxicol.*, 9, 397-406, 1990.

McCarver, D.G., Hines, R.N. 2002. The ontogeny of human drug-metabolizing enzymes: phase II conjugation enzymes and regulatory mechanisms. *J. Pharmacol. Exp. Ther.* 300(2), 361-6.

Medinsky, M.A. 1995. The application of physiologically based pharmacokinetic/pharmacodynamic (PBPK/PD) modeling to understanding the mechanism of action of hazardous substances. *Toxicol. Lett.* 79, 185-191.

Meek, M.E., Renwick, A., Ohanian, E., Dourson, M., Lake, B., Naumann, B.D., Vu, V., International Programme on Chemical Safety (IPCS). 2002. Guidelines for application of chemical-specific adjustment factors in dose/concentration-response assessment. *Toxicology.* 181-182,115-20.

Menzel, D.B., Wolpert, R.L., Boger, J.R., Kootsey, J.M. 1987. Resources available for simulation in toxicology: specialized computers, generalized software and communication networks. *Drinking Water and Health.* 8, 229-254.

Nakajima, T., Elovaara, E., Park, S.S., Gelboin, H.V., Vainio, H. 1991. Immunochemical detection of cytochrome P450 isozymes induced in rat liver by n-hexane, 2-hexanone and acetonyl acetone. *Arch Toxicol.* 65, 542-7.

Nakajima, T., Wang, R.S. 1994. Induction of cytochrome P450 by toluene. *Int. J. Biochem.* 26, 1333-40.

Nakajima, T., Wang, R.S., Elovaara, E., Gonzalez, F.J., Gelboin, H.V., Raunio, H., Pelkonen, O., Vainio, H., Aoyama, T. 1997. Toluene metabolism by cDNA-expressed human hepatic cytochrome P450. *Biochem. Pharmacol.* 53,271-7.

Nakamura, H., Tanaka, E., Ishikawa, A., Fukao, K., Tsuji, K., Ohkawa, H. 1998. Age-related changes in hepatic drug-oxidizing activity using trimethadione as a probe drug in humans. *Hepatol. Res.* 12, 85–92.

National Toxicology Program (NTP). 1990. *Toxicology and carcinogenesis studies of toluene (CAS No. 108-88-3) in F344/N rats and B5C3F1 mice (inhalation studies)*. Public Health Service, U.S. Department of Health and Human Services; NTP TR 371.

Nihlen, A., Lof, A., Johanson, G. 1995. Liquid/air partition coefficients of methyl and ethyl T-butyl ethers, T-amyl methyl ether, and T-butyl alcohol. *J. Expo. Anal. Environ. Epidemiol.* 5,573-82.

Nihlen, A., Lof, A., Johanson, G. 1998. Experimental exposure to methyl tertiary-butyl ether. I. Toxicokinetics in humans. *Toxicol. Appl. Pharmacol.* 148,274-80.

Nishimoto, I.N., Hanaoka, T., Sugimura, H., Nagura, K., Ihara, M., Li, X.J., Arai, T., Hamada, G.S., Kowalski, L.P., Tsugane, S. 2000. Cytochrome P450 2E1 polymorphism in gastric cancer in Brazil: case-control studies of Japanese Brazilians and non-Japanese Brazilians. *Cancer Epidemiol. Biomarkers Prev.* 9(7), 675-80.

Nong, A., McCarver, D.G., Hines, R.N., Krishnan, K. 2006. Modeling interchild differences in pharmacokinetics on the basis of subject-specific data on physiology and hepatic CYP2E1 levels: a case study with toluene. *Toxicol. Appl. Pharmacol.* 214, 78-87.

O'Flaherty, E.J. 1998. A physiologically based kinetic model for lead in children and adults. *Environ. Health Perspect.* 106 Suppl 6, 1495-503.

O'Flaherty, E.J. 1998. PB/PK models. In: W. Slikker, L.W. Chang. Eds. *Handbook of Developmental Neurotoxicology.* Academic Press, San Diego, CA. p. 307-320.

Ogata, M. 1984. Estimation of solvent concentrations in ambient air from urinary metabolite levels of workers exposed to solvents. *Ind. Health.* 22, 319-24.

Payne, M.P, Kenny, L.C. 2002. Comparison of models for the estimation of biological partition coefficients. *J. Toxicol. Environ. Health A.* 65,897-931.

Pelekis, M., Gephart, L.A., Lerman, S.E. 2001. Physiological-model-based derivation of the adult and child pharmacokinetic intraspecies uncertainty factors for volatile organic compounds. *Regul. Toxicol. Pharmacol.* 33(1), 12-20.

Pelekis, M., Krewski, D., Krishnan, K. 1997. Physiologically based algebraic expressions for predicting steady-state toxicokinetics of inhaled vapors. *Toxicol. Meth.* 7, 205-225.

Pelekis, M., Nicolich, M.J., Gauthier, J.S. 2003. Probabilistic framework for the estimation of the adult and child toxicokinetic intraspecies uncertainty factors. *Risk Anal.* 23(6), 1239-55.

Pierce C.H., Dills R.L., Silvey G.W., Kalman D.A. 1996. Partition coefficients between human blood or adipose tissue and air for aromatic solvents. *Scand. J. Work. Environ. Health.* 22, 112-8.

Portier, C.J., Kaplan, N.L. 1989. Variability of safe dose estimates when using complicated models of the carcinogenic process. A case study: methylene chloride. *Fundam. Appl. Toxicol.* 13, 533-44.

Poulin, P, Krishnan, K. 1995. A biologically-based algorithm for predicting human tissue: blood partition coefficients of organic chemicals. *Hum. Exp. Toxicol.* 14, 273-280.

Poulin, P, Krishnan, K. 1996c. Molecular structure based prediction of the partition coefficients of organic chemicals for physiological pharmacokinetic models. *Toxicol. Methods.* 6, 117-137.

Poulin, P., Krishnan, K. 1996a. A tissue composition-based algorithm for predicting tissue: air partition coefficients of organic chemicals. *Toxicol. Appl. Pharmacol.* 136, 126-130.

Poulin, P., Krishnan, K. 1996b. A mechanistic algorithm for predicting blood: air partition coefficients of organic chemicals with the consideration of reversible binding in haemoglobin. *Toxicol. Appl. Pharmacol.* 136, 131-137.

Poulin, P., Krishnan, K. 1998. A quantitative structure-toxicokinetic relationship model for highly metabolised chemicals. *Altern. Lab. Anim.* 26, 45-59.

Poulin, P., Krishnan, K. 1999. Molecular structure-based prediction of the toxicokinetics of inhaled vapors in humans. *Int. J. Toxicol.* 18, 7-18.

Prah, J., Ashley, D., Blount, B., Case, M., Leavens, T., Pleil, J., Cardinali, F. 2004. Dermal, oral, and inhalation pharmacokinetics of methyl tertiary butyl ether (MTBE) in human volunteers. *Toxicol. Sci.* 77,195-205.

Price K., Haddad S., Krishnan K. 2003a. Physiological modeling of age-specific changes in the pharmacokinetics of organic chemicals in children. *J. Toxicol. Environ. Health A.* 66, 417-33.

Price, P.S., Conolly, R.B., Chaisson, C.F., Gross, E.A., Young, J.S., Mathis, E.T., Tedder, D.R. 2003b. Modeling interindividual variation in physiological factors used in PBPK models of humans. *Crit Rev Toxicol.* 33, 469-503.

Ramsey, J.C, Andersen, M.E. 1984. A physiologically-based description of the inhalation pharmacokinetics of styrene in rats and humans. *Toxicol. Appl. Pharmacol.*, 73, 159-175.

Rane, A., Wilkinson, G.R., Shand, D.G. 1977. Prediction of hepatic extraction ratio from in vitro measurement of intrinsic clearance. *J. Pharmacol. Exp. Ther.* 200, 420-424.

Rao, H.V., Ginsberg, GL. 1997. A physiologically-based pharmacokinetic model assessment of methyl t-butyl ether in goundwater for a bathing and showering determination. *Risk. Anal.* 17,583-598.

Reddy, M., Yang, R.S., Andersen, M.E., Clewell, H.J. 2005. *Physiological based pharmacokinetic modeling: science and applications.* John Wiley & Sons, Inc., Hoboken, NJ.

Reitz., R.H., Gargas, M.L., Mendrala, A.L., Schumann, A.M. 1996. In vivo and in vitro studies of perchloroethylene metabolism for physiologically based pharmacokinetic modeling in rats, mice, and humans. *Toxicol. Appl. Pharmacol.* 136, 289-306.

Renwick, A.G. 1993. Data-derived safety factors for the evaluation of food additives and environmental contaminants. *Food Addit. Contam.* 10, 275-305.

Renwick, A.G., Lazarus, N.R. 1998. Human variability and noncancer risk assessment--an analysis of the default uncertainty factor. *Regul. Toxicol. Pharmacol.* 27, 3-20.

Rodman, J.H., Relling, M.V., Stewart, C.F., Synold, T.W., McLeod, H., Kearns, C., Stute, N., Crom, W.R., Evans, W.E. 1993. Clinical pharmacokinetics and pharmacodynamics of anticancer drugs in children. *Semin. Oncol.* 20(1), 18-29.

Rumchev, K., Spickett, J., Bulsara, M., Phillips, M., Stick, S. 2004. Association of domestic exposure to volatile organic compounds with asthma in young children. *Thorax.* 59, 746-751.

Sato, A. Nakajima, T. 1979a. A vial equilibration method to evaluate the drug metabolizing enzyme activity for volatile hydrocarbons. *Toxicol. Appl. Pharmacol.* 47, 41-46.

Sato, A, Nakajima, T. 1979b. Partition coefficients of some aromatic hydrocarbons and ketones in water, blood and oil. *Br. J. Ind. Med.*, 36, 231-234.

Sexton, K., Adgate, J.L., Church, T.R., Ashley, D.L., Needham, L.L., Ramachandran, G., Frederickson, A.L., Ryan, A.D. 2005. Children,s exposure to volatile organic compounds as determined by longitudinal measurements in blood. *Environ. Health Perspect.* 113, 342-349.

Shah, R.R. 2004. Drug development and use in the elderly: search for the right dose and dosing regimen (Parts I and II). *Br. J. Clin. Pharmacol.* 58(5), 452-69.

Shelley, M.L., Andersen, M.E., Fisher, J.W. 1988. An inhalation distribution model for the lactating mother and nursing child. *Toxicol Lett.* 43(1-3), 23-9.

Smith, T.J., Lin, Y.S., Mezzetti, M., Bois, F.Y., Kelsey, K., Ibrahim, J. 2001. Genetic and dietary factors affecting human metabolism of 1,3-butadiene. *Chem. Biol. Interact.* 135-136, 407-28.

Snawder, J.E., Lipscomb, J.C. 2000. Interindividual variance of cytochrome P450 forms in human hepatic microsomes: correlation of individual forms with xenobiotic metabolism and implications in risk assessment. *Regul. Toxicol. Pharmacol.* 32(2), 200-9.

Sohn, M.D., McKone, T.E., Blancato, J.N. 2004. Reconstructing population exposures from dose biomarkers: inhalation of trichloroethylene (TCE) as a case study. *J. Expo. Anal. Environ. Epidemiol.* 14(3), 204-13.

Sonnier, M., Cresteil, T. 1998. Delayed ontogenesis of CYP1A2 in the human liver. *Eur. J. Biochem.* 251, 893-8.

Spear, R.C., Bois, F.Y. 1994. Parameter variability and the interpretation of physiologically based pharmacokinetic modeling results. *Environ. Health Perspect.* 102 Suppl 11, 61-6.

Stevens J.C., Hines R.N., Gu C., Koukouritaki S.B., Manro J.R., Tandler P.J., Zaya M.J. 2003. Developmental expression of the major human hepatic CYP3A enzymes. *J. Pharmacol. Exp. Ther.* 307, 573-82.

Swartout, J.C., Price, P.S., Dourson, M.L., Carlson-Lynch, H.L., Keenan, R.E. 1998. A probabilistic framework for the reference dose (probabilistic RfD). *Risk Anal.* 18(3), 271-82.

Sweeney, L.M., Kirman, C.R., Morgott, D.A., Gargas, M.L. 2004. Estimation of interindividual variation in oxidative metabolism of dichloromethane in human volunteers. *Toxicol Lett.* 154, 201-16.

Sweeney, L.M., Tyler, T.R., Kirman, C.R., Corley, R.A., Reitz, R.H., Paustenbach, D.J., Holson, J.F., Whorton, M.D., Thompson, K.M., Gargas, M.L. 2001. Proposed occupational exposure limits for select ethylene glycol ethers using PBPK models and Monte Carlo simulations. *Toxicol. Sci.* 62(1), 124-39.

Syme, M.R., Paxton, J.W., Keelan, J.A. 2004. Drug transfer and metabolism by the human placenta. *Clin Pharmacokinet.* 43, 487-514.

Tanaka, E. 1998. Clinically important pharmacokinetic drug-drug interactions: Role of cytochrome P450 enzymes. *J. Clin. Pharm. Ther.* 23, 403–416.

Tardif, R., Charest-Tardif, G., Brodeur, J., Krishnan, K. 1997. Physiologically based pharmacokinetic modeling of a ternary mixture of alkyl benzenes in rats and humans. *Toxicol. Appl. Pharmacol.* 144, 120-134.

Tardif, R., Droz, P.O., Charest-Tardif, G., Pierrehumbert, G., Truchon, G. 2002. Impact of human variability on the biological monitoring of exposure to toluene: I. Physiologically based toxicokinetic modelling. *Toxicol Lett.* 134(1-3), 155-63.

Tardif, R., Lapare, S., Charest-Tardif, G., Brodeur, J., Krishnan, K. 1995. Physiologically-based pharmacokinetic modeling of a mixture of toluene and xylene in humans. *Risk Anal.* 15, 335-342.

Tardif, R., Lapare, S., Krishnan, K., Brodeur, J., 1993. Physiologically based modeling of the toxicokinetic interaction between toluene and m-xylene in the rat. *Toxicol. Appl. Pharmacol.* 120, 266-73.

Tardif, R., Truchon, G., Brodeur, J. 1998. Comparison of hippuric acid and o-cresol in urine and unchanged toluene in alveolar air for the biological monitoring of exposure to toluene in human volunteers. *Appl. Occup. Environ. Hyg.* 13, 127-132.

Tassaneeyakul, W., Birkett, D.J., Edwards, J.W., Veronese, M.E., Tassaneeyakul, W., Tukey, R.H., Miners, J.O. 1996. Human cytochrome P450 isoform specificity in the regioselective metabolism of toluene and o-, m- and p-xylene. *J. Pharmacol. Exp. Ther.* 276, 101-8.

Thomas, R.S., Bigelow, P.L., Keefe, T.J., Yang, R.S. 1996. Variability in biological exposure indices using physiologically based pharmacokinetic modeling and Monte Carlo simulation. *Am. Ind. Hyg. Assoc. J.* 57, 23-32.

Thomas, R.S., Lytle, W.E., Keefe, T.J., Constan, A.A., Yang, R.S. 1996. Incorporating Monte Carlo simulation into physiologically based pharmacokinetic models using advanced continuous simulation language (ACSL): a computational method. *Fundam. Appl. Toxicol.* 31(1), 19-28.

Timchalk, C., Kousba, A., Poet, T.S. 2002. Monte Carlo analysis of the human chlorpyrifos-oxonase (PON1) polymorphism using a physiologically based pharmacokinetic and pharmacodynamic (PBPK/PD) model. *Toxicol Lett.* 135(1-2), 51-59.

US Environmental Protection Agency (USEPA). 1994. *Methods for derivation of inhalation reference concentrations and application of inhalation dosimetry.* USEPA, Washington, DC, EPA/600/8-90/066F.

US Environmental Protection Agency (USEPA). 1996. *Summary Report for the Workshop on Monte Carlo Analysis.* U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC, EPA/630/R-96/010.

US Environmental Protection Agency (USEPA). 1997a. *Exposure Factors Handbook.* USEPA, Office of Research and Development, National Center for Environmental Assessment, Washington Office, Washington, DC. EPA/600/P-95/002.

US Environmental Protection Agency (USEPA). 1997b. *Guiding Principles for Monte Carlo Analysis.* U.S. Environmental Protection Agency, Risk Assessment Forum, Washington, DC, EPA/630/R-97/001.

US Environmental Protection Agency (USEPA). 2001. *Risk Assessment Guidance for Superfund Volume 3 Part A: Process for Conducting Probabilistic Risk Assessment (RAGS 3A).* USEPA, Office of Research and Development, National Center for Environmental Assessment, Washington, DC. EPA/540/R-02/002.

US Environmental Protection Agency (USEPA). 2002. *Child-Specific Exposure Factors Handbook (Interim Report) 2002.* USEPA, Office of Research and Development, National Center for Environmental Assessment, Washington, DC. EPA-600-P-00-002B.

US Environmental Protection Agency (USEPA). 2005. Toxicological review of toluene in support of summary information on the Integrated Risk Information System (IRIS). USEPA, Office of Research and Development, National Center for Environmental Assessment, Washington, DC. EPA/635/R-05/004.

Vieira, I., Sonnier, M., Cresteil, T. 1996. Developmental expression of CYP2E1 in the human liver. Hypermethylation control of gene expression during the neonatal period. *Eur. J. Biochem.* 238, 476–483.

Vinegar, A., Jepson, G.W., Cisneros, M., Rubenstein, R., Brock, W.J. 2000. Setting safe acute exposure limits for halon replacement chemicals using physiologically based pharmacokinetic modeling. *Inhal. Toxicol.* 12(8), 751-63.

Wagner, J.G. 1975. *Fundamentals of Clinical Pharmacokinetics*, Drug International. Hamilton, IL.

Wakefield, J. 1996. Bayesian individualization via sampling-based methods. *J. Pharmacokinet. Biopharm.* 24(1), 103-31.

Wallace, L., Nelson, W., Ziegenfus, R., Pellizzari, E., Michael, L., Whitmore, R., Zelon, H., Hartwell, T., Perritt, R., Westerdahl, D. 1991. The Los Angeles TEAM Study: personal exposures, indoor-outdoor air concentrations, and breath concentrations of 25 volatile organic compounds. *J. Expo. Anal. Environ. Epidemiol.* 1(2), 157-92.

Wallace, L.A. 1991. Comparison of risks from outdoor and indoor exposure to toxic chemicals. *Environ. Health Perspect.* 95, 7-13.

White, D.R., Widdowson, E.M., Woodard, H.Q. 1991. The composition of body tissues. (II) Fetus to young adult. *Br. J. Radiol.* 64, 149–159.

Wilkinson, G.R., Shand, D.G. 1975. Commentary: a physiological approach to hepatic drug clearance. *Clin. Pharmacol. Ther.* 18, 377-90.

Woodruff, T.J., Bois, F.Y., Auslander, D., Spear, R.C. 1992. Structure and parameterization of pharmacokinetic models: their impact on model predictions. *Risk Anal.* 12(2), 189-201.

Young, J.F., Branham, W.S., Sheehan, D.M., Baker, M.E., Wosilait, W.D., Luecke, R.H. 1997. Physiological "constants" for PBPK models for pregnancy. *J. Toxicol. Environ. Health.* 52(5), 385-401.