Université de Montréal

Évaluation de la liaison des substances organiques volatiles aux protéines du sang chez le rat

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Évaluation de la liaison des substances organiques volatiles aux protéines du sang chez le rat

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a été évalué par un jury composé des personnes suivantes:

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Le coefficient de partage (CP) sang:air (P_b) est un déterminant important de la pharmacocinétique des substances organiques volatiles (SOV). Présentement, les algorithmes qui permettent de prédire la valeur des P_b des SOV en considérant la solubilité d'une substance dans les fractions lipidiques et aqueuses du sang ne tiennent pas compte de la liaison potentielle des SOV aux protéines sanguines (PS). Ce projet de recherche visait à évaluer la liaison des SOV aux PS du rat dans des conditions *in vitro*. Les objectifs étaient: i) d'identifier les caractéristiques communes aux SOV pouvant être potentiellement impliquées dans la liaison aux PS, ii) de vérifier expérimentalement la présence de la liaison des SOV aux PS du rat, iii) d'étudier l'implication de l'hémoglobine (Hb) dans cette liaison, iv) de déterminer l'impact de la concentration d'exposition sur le P_b, et, v) de déterminer la valeur de la constante d'affinité (K_a) et le nombre de sites de liaisons (n) des protéines impliquées. Dans un premier temps, les caractéristiques des SOV identifiés dans la littérature comme étant théoriquement liés aux PS chez le rat ont été analysées afin d'identifier certains points communs. Ainsi, des caractéristiques portant sur la taille moléculaire (>300 Å), la lipophilicité (log CP *n*-octanol:eau ≥ 1), et la composition élémentaire des SOV (aucun oxygène) ont été identifiées comme étant des éléments importants de la liaison aux protéines. Dans un deuxième temps, en se basant sur ces caractéristiques communes, l'occurence de liaison a été prédite pour certains SOV. Par la suite, nous avons procédé à une validation expérimentale qui consistait à comparer les CP entre l'air et un mélange huile+eau et le CP entre l'air et le sang de rat. Les résultats ont montré que les CP sang:air étaient significativement supérieurs aux CP mélange:air (moyenne±SE; n=3-8)

pour le bromoforme (BF; 161±5 vs 28.9±3.4), chlorobenzène (CB; 61.8±2.8 vs 8.3±2.35), chloroforme (CF; 16.9±1.1 vs 3.4±0.75), et éthylbenzène (EB; 50.8±1.3 vs 7.13±1.6). Cela suggérait fortement l'existence de liaisons aux PS. L'implication de l'Hb dans la liaison a également été évaluée puisque que l'Hb etait soupconnée être la principale protéine liante. Les CP reconstitution de sang (*n*-octanol+eau+Hb):air et les CP sang:air ont été déterminés. Les résultats ont démontré que la liaison à l'Hb jouait un rôle majeur dans la valeur du P_b pour les SOV étudiés. En effet, la valeur du CP du mélange *n*-octanol+eau+Hb était proche de la valeur du P_b réel pour ces substances [BF (154±1.5), CB (55±6), CF (15±0.87), et EB (30±1.5)]. Dans un troisième temps, les valeurs de K_a (mM^{-1}) et de n, ainsi que l'impact de la concentration d'exposition sur le P_b ont été déterminés. L'utilisation de fortes concentrations de SOV s'est traduite par une diminution de P_b, ce qui suggère une saturation des sites de liaison. Les valeurs expérimentales de K_a et de n sont les suivantes: BF (0.8, 4), CB (2.8, 1.4), CF (1.8, 1.2), et EB (2, 1.4). Cette étude représente la première tentative visant à caractériser et prédire la liaison des SOV aux PS chez le rat, à étudier ses conséquences sur la cinétique des substances et à inclure cette caractéristique dans la structure des modèles pharmacocinétique à base physiologique utilisés pour l'analyse du risque toxicologique.

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LISTE DES SIGLES ET DES ABRÉVIATIONS

ACSL®	Advanced Continuous Simulation Language®
AUC	surface sous la courbe
BF	bromoform
BME	butyl methyl ether
C_a	concentration artérielle (mg/L)
$C_{a,bound}$	concentration de substance liée dans le sang (mM)
$C_{a,free}$	concentration de substance non-liée dans le sang (mM)
C_{air}	concentration atmosphérique de substance (mM)
C_{alv}	concentration de la substance dans l'air alvéolaire (mg/L)
$C_{a(total)}$	concentration totale de substance (mM)
CB	chlorobenzene
C_b	concentration de substance liée (mM)
CF	chloroform
C_f	concentration de la substance dans le tissu adipeux (mg/L)
C_{hs}	concentration de substance dans l'espace de tête du tube (mM)
C_{inh}, C_i	concentration inhalée ou environmentale (mg/L)
C_l	concentration de la substance dans le foie (mg/L)
CL_h	clairance hépatique (L/h)
CL_{int}	clairance intrinsèque (L/h)
CO_2	dioxyde de carbone
C_p	concentration de protéines dans le sang (mM)
C_{pu}	concentration de protéines non-liée (mM)

C_r	concentration de la substance dans les tissus richement perfusés (mg/L); concentration de substance dans le tube de référence (mg/L)
C_s	concentration de la substance dans les tissus faiblement perfusés (mg/L); concentration de substance dans l'espace de tête du tube qui contient l'échantillon (mg/L)
C_t, C_{tot}	concentration de la substance dans le tissu (mg/L); concentration totale de substance (mM)
C_u, C_{free}	concentration de substance non-liée (mM)
C_v	concentration veineuse (mg/L)
C_{vf}	concentration veineuse quittant le tissu adipeux (mg/L)
C_{vl}	concentration veineuse quittant le foie (mg/L)
C_{vr}	concentration veineuse quittant les tissus richement perfusés (mg/L)
C_{vs}	concentration veineuse quittant les tissus faiblement perfusés (mg/L)
C_{vt}	concentration veineuse quittant le tissu (mg/L)
$\delta A_L/\delta t$	taux de changement de la quantité de substance dans les poumons (mg/h)
$\delta A_{mc}/\delta t$	taux de changement de la quantité de substance dans la matrice cellulaire (mg/h)
$\delta A_{met}/\delta t$	taux de changement de la quantité de substance métabolisée (mg/h)
$\delta A_{st}/\delta t$	taux de changement de la quantité de substance dans le sang (mg/h)
$\delta A_t/\delta t$	taux de changement de la quantité de substance dans le tissu (mg/h)
$\delta C_f/\delta t$	taux de changement de la concentration de la substance dans le tissu adipeux (mg/L/h)

$\delta C_l/\delta t$	taux de changement de la concentration de la substance dans le foie (mg/L/h)
$\delta C_r/\delta t$	taux de changement de la concentration de la substance dans les tissus richement perfusés (mg/L/h)
$\delta C_s/\delta t$	taux de changement de la concentration de la substance dans les tissus faiblement perfusés (mg/L/h)
D_t	constante de diffusion tissulaire (L/h)
E	coefficient d'extraction hépatique
EB	ethylbenzene
ETH	diethyl ether
GV	volume géométrique ($Å^3$)
Hb	hémoglobine
hr	heure
ISO	isooctane
K_a	constante d'affinité pour les protéines du sang (mM^{-1})
K_f	constante métabolique de premier ordre (h^{-1})
K_m	constante d'affinité Michaëlis-Menten (mg/L)
Log $P_{(o:w)}$	log coefficient de partage *n*-octanol:eau
MEK	methyl ethyl ketone
n	nombre d'échantillons; nombre de sites de liaison
P_b, $P_{b:a}$	coefficient de partage sang:air
$P_{b:a(app)}$	coefficient de partage sang:air apparent
PBPK	pharmacocinétique à base physiologique (identique à PCBP)
PC	coefficient de partage

PCBP	pharmacocinétique à base physiologique (identique à PBPK)
P_f	coefficient de partage tissu adipeux:sang
PIN	α-pinene
PK	pharmacocinétique
$P_{l:a}$	coefficient de partage lipide:air
P_l	coefficient de partage foie:sang
$P_{o:a}$	coefficient de partage huile végétale:air (ou *n*-octanol:air)
$P_{o:w}$	coefficient de partage *n*-octanol:eau
$P_{o+w:a}$	coefficient de partage *n*-octanol+eau:air
P_r	coefficient de partage tissus richement perfusés:sang
P_s	coefficient de partage tissus faiblement perfusés:sang
$P_{t:a}$	coefficient de partage tissu:air
P_t, $P_{t:b}$	coefficient de partage tissu:sang
$P_{w:a}$	coefficient de partage eau:air
Q_c	débit cardiaque (L/h)
Q_f	débit sanguin au tissu adipeux (L/h)
Q_l	débit sanguin au foie (L/h)
Q_p	taux de ventilation alvéolaire (L/h)
Q_r	débit sanguin au tissu richement perfusés (L/h)
Q_s	débit sanguin au tissus faiblement perfusés (L/h)
Q_t	débit sanguin au tissu (L/h)
r	ratio molaire de substance liée
RAM	taux de métabolisme hépatique (mg/h)
SOV	substance(s) organique(s) volatile(s) (identique à VOCs)

t	intervalle de temps
tBME	*t*-butyl methyl ether
V_f	volume du tissu adipeux (L)
V_l	volume du foie (L)
V_{lb}	fraction du volume sanguin correspondant aux lipides
V_{lt}	fraction du volume tissulaire correspondant aux lipides
V_{max}	vitesse maximale de biotransformation d'un composé (mg/h)
V_{nb}	fraction du volume sanguin correspondant aux lipides neutres
V_{nt}	fraction du volume tissulaire correspondant aux lipides neutres
VOCs	substances organiques volatiles (identique à SOV)
V_{pb}	fraction du volume sanguin correspondant aux phospholipides
V_{pt}	fraction du volume tissulaire correspondant aux phopholipides
V_r	volume des tissus richement perfusés (L); volume du tube de référence (ml)
V_s	volume des tissus faiblement perfusés (L); volume de l'échantillon (ml)
V_t	volume du tissu (L)
V_{wb}	fraction du volume sanguin correspondant à l'eau
V_{wt}	fraction du volume tissulaire correspondant à l'eau

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CHAPITRE PREMIER :

1 - INTRODUCTION GÉNÉRALE

1. INTRODUCTION

1.1 La modélisation pharmacocinétique à base physiologique

La modélisation pharmacocinétique à base physiologique (PCBP) consiste à décrire mathématiquement l'absorption, la distribution, le métabolisme et l'élimination de substances dans l'organisme. Les équations différentielles qui y sont incluses sont basées sur la physiologie de l'organisme, ainsi que sur les caractéristiques physico-chimiques des substances. Ces équations permettent la prédiction de la cinétique des substances dans l'organe cible (Krishnan et Andersen, 1994). Les relations entre les paramètres qui servent d'intrants dans ces équations, c-à-d, les paramètres physiologiques (débit alvéolaire, débit cardiaque, volumes tissulaires et débits sanguins aux tissus), physico-chimiques (coefficients de partage, coefficients de diffusion, et constantes d'absorption), et biochimiques (taux d'élimination, constantes de liaisons, et métabolisme) déterminent le stockage et le devenir d'une substance dans un organisme.

Les modèles PCBP permettent la prédiction de la concentration sanguine ou tissulaire d'une substance à partir de la dose d'exposition. Puisque la toxicité d'une substance est considérée comme étant directement liée à sa concentration tissulaire (Andersen *et al.*, 1987), ces modèles sont utilisés de plus en plus fréquemment en analyse du risque toxicologique. Ainsi, grâce à la modélisation PCBP, la détermination de la concentration d'une substance dans l'organe cible en fonction du temps et de la concentration d'exposition est possible. De plus, en étant une représentation mathématique de mécanismes biologiques, plusieurs phénomènes observés dans un organisme, tel que la saturation des processus de

biotransformation, peuvent y être représentés, et leur influence sur la concentration tissulaire peut être facilement déterminée.

L'utilisation des modèles PCBP en analyse du risque toxicologique présente certains avantages. Premièrement, puisqu'ils sont constitués de mécanismes biologiques qui facilitent le calcul de la concentration interne, leur utilisation permet de relier celle-ci plutôt que la dose d'exposition à l'effet observé, ce qui reduit l'incertitude reliée aux méthodes traditionnelles d'extrapolation des doses d'expositions. Ainsi, le scénario d'exposition, la voie d'absorption, la dose et l'espèce peuvent être considérés lors d'extrapolations (Krishnan et Andersen, 1994). Deuxièmement, si la modélisation PCBP est combinée à des études toxicocinétiques chez l'animal, les mécanismes responsables de la toxicocinétique d'une substance donnée peuvent être mieux compris. En effet, dans le cas où le profil cinétique généré par le modèle PCBP ne correspond pas aux données déterminées expérimentalement, d'autres processus biologiques devront être incorporés dans le modèle ou certains paramètres du modèle devront être modifiés afin de refléter la réalité expérimentale (Clewell et Andersen, 1987; Haddad *et al.*, 1998). Les hypothèses qui sont générées ainsi peuvent être ensuite vérifiées expérimentalement.

Le développement d'un modèle PCBP se fait en quatre étapes (Figure 1). D'abord le modèle est représenté d'une façon conceptuelle et fonctionnelle. Ensuite, les paramètres du modèle sont estimés. Puis, la cinétique de la substance est simulée. Finalement, les résultats de la simulation sont validés et le modèle est mis au point. La représentation du modèle (Fig. 1) est l'étape où l'on développe la description conceptuelle et mathématique des divers compartiments, et où l'on

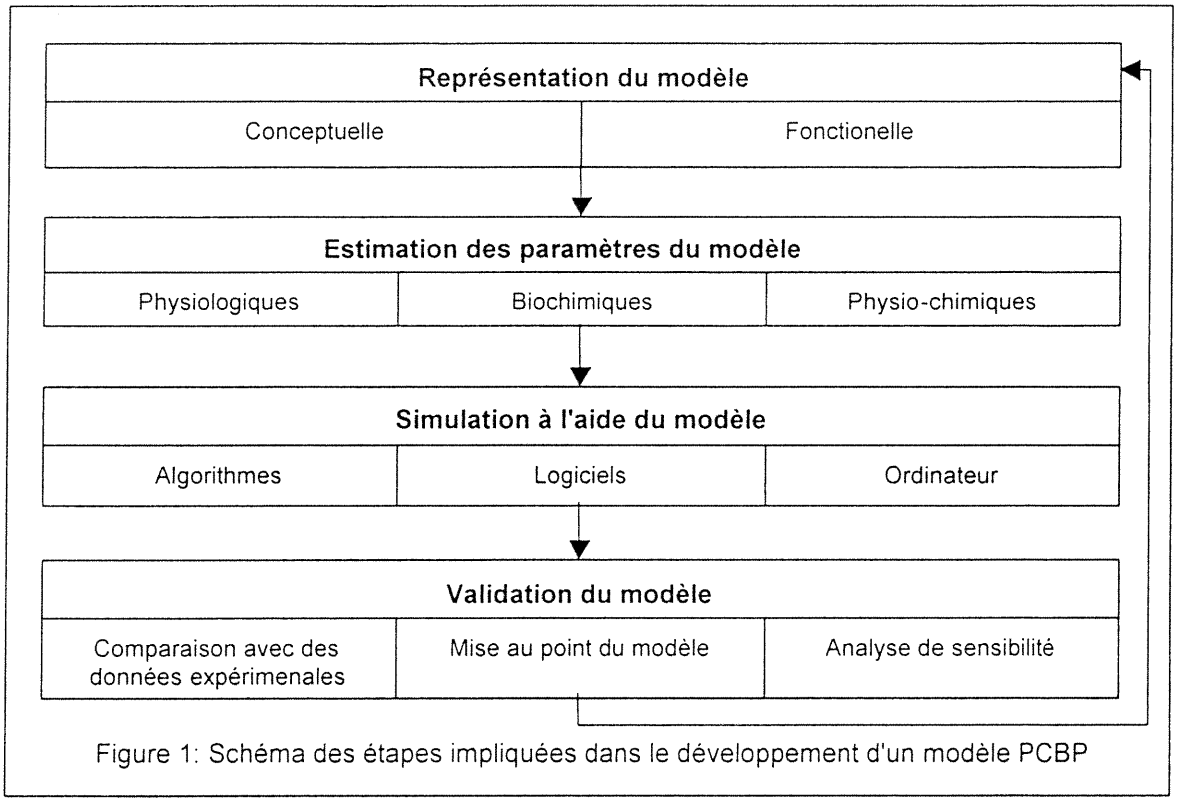

Figure 1: Schéma des étapes impliquées dans le développement d'un modèle PCBP

identifie les voies d'exposition et les processus métaboliques de la substance chez l'animal. L'étape d'estimation des paramètres est caractérisée par la détermination, d'une façon indépendante, de la valeur numérique des différents paramètres (physiologiques, physicochimiques, et biochimiques) qui serviront d'intrants dans les équations du modèle. Lors de l'étape de la simulation, la solution des équations différentielles du modèle est déterminée selon une méthode numérique appropriée (p. ex., Gear, Runge-Kutta, Euler) (Krishnan et Andersen, 1994; Haddad *et al.*, 1996) et en utilisant un logiciel de simulation (p. ex., ACSL®, ScoP®, CSSL IV®, SIMNON®, ADSIM® et STELLA®), un logiciel de programmation (p. ex., Fortran ou BASIC), ou un tabulateur (p. ex., EXCEL®) (Haddad *et al.*, 1996). La dernière étape est celle de la validation, où la capacité des représentations du modèle PCBP à prédire la cinétique des substances est assurée. La validation se fait le plus souvent par inspection, une méthode qui consiste en une comparaison

visuelle des données prédites et expérimentales en fonction d'une variable commune (p. ex., temps). Elle peut se faire aussi par mesure de divergence, une méthode qui consiste à quantifier la différence observée entre les données simulées et experimentales (Krishnan et Pelekis, 1995). Il est aussi possible de valider le modèle PCBP statistiquement, bien que l'utilisation de cette approche ne fasse que commencer (Krishnan et Andersen, 1994; Haddad *et al.* 1995). La validation déterminera si la description d'un modèle est appropriée ou si des mises au point du modèle sont nécessaires afin que la simulation représente mieux les données expérimentales. Lorsqu'un modèle est validé, l'extrapolation de la cinétique d'une substance en fonction du scénario d'exposition, de la dose administrée, ou de l'espèce étudiée est possible. La première étape du développement d'un modèle PCBP consiste donc à représenter le modèle d'une façon conceptuelle et fonctionnelle.

1.1.1 Représentation du modèle

1.1.1.1 Représentation conceptuelle

La représentation conceptuelle d'un modèle PCBP est l'étape ou une représentation schématique de l'organisme est développée et où l'on identifie les tissus responsables de la cinétique selon le scénario d'exposition, les caractéristiques physiologiques des tissus, ainsi que les caractéristiques toxicologiques, physico-chimiques, et biochimiques de la substance (Krishnan et Andersen, 1994). Afin de développer un modèle PCBP pour une substance quelconque, les points suivants devront être considérés : 1) l'organe cible, 2) la ou

les voies d'absorption, 3) le ou les tissus où il y a évidence de distribution, métabolisme et/ou élimination, et 4) le ou les tissus où le débit sanguin, le volume ou la composition peuvent mener à une accumulation de la substance.

Les tissus contribuant à l'absorption, à la distribution, au métabolisme, à l'élimination, et à la toxicité de la substance sont ainsi groupés sous forme de compartiments, et les tissus restants sont groupés selon leur composition. Conséquemment, un modèle PCBP pour une substance organique volatile (SOV) relativement lipophile peut être représenté conceptuellement par le foie (tissu métabolisant), un compartiment représentant les poumons (où l'absorption à lieu), un compartiment représentant les tissus adipeux (où il y a accumulation de substance), un compartiment représentant l'ensemble des tissus faiblement perfusés, et un compartiment représentant l'ensemble des tissus rapidement perfusés. Les tissus ainsi considérés par le modèle ne représentent que 91% du poids corporel, puisque les tissus restants représentant le 9% (p. ex., os, ongles et poils) sont peu irrigués et ont ainsi peu d'influence sur la cinétique des SOV. Un exemple de représentation conceptuelle d'un modèle PCBP est

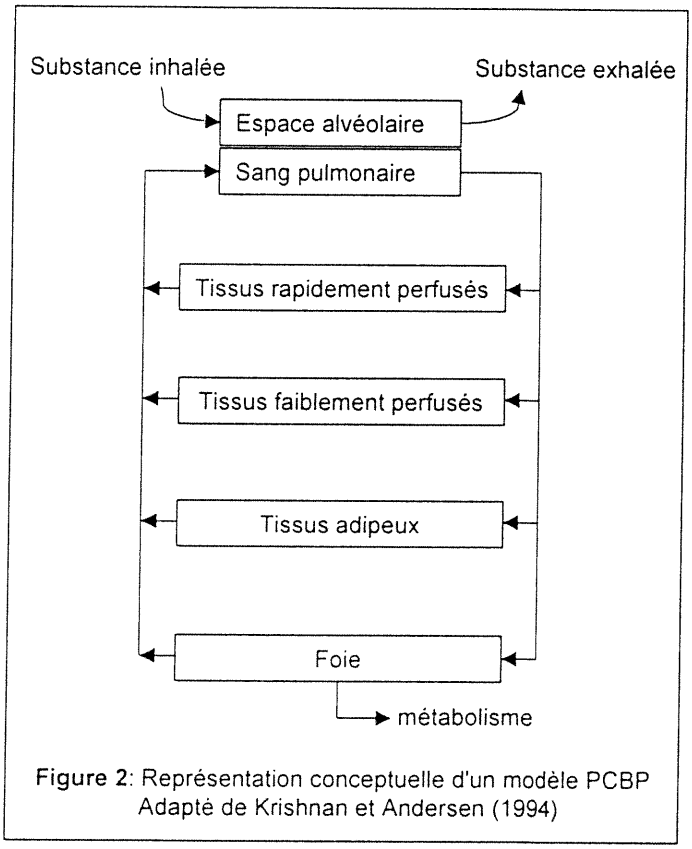

Figure 2: Représentation conceptuelle d'un modèle PCBP
Adapté de Krishnan et Andersen (1994)

présenté à la Figure 2. Suite à cette représentation conceptuelle, le modèle doit ensuite être représenté de façon fonctionnelle.

1.1.1.2 Représentation fonctionnelle

La représentation fonctionnelle d'un modèle PCBP est l'étape où les processus physiologiques, physico-chimiques, et biochimiques qui ont lieu dans chaque compartiment, ainsi que les liens entre ces compartiments tissulaires, sont décrits mathématiquement.

DESCRIPTION MATHÉMATIQUE DES COMPARTIMENTS TISSULAIRES

Chaque compartiment tissulaire est décrit mathématiquement par une équation différentielle de bilan de masse, où le taux de changement de la quantité d'une substance dans le compartiment est fonction du taux de ce qui entre et du taux de ce qui quitte le compartiment. Selon le poids moléculaire de la substance, l'équation du bilan de masse peut refléter un processus de distribution dans le tissu qui est limité par la diffusion du produit dans la matrice cellulaire, ou alors par la perfusion sanguine (Krishnan et Andersen, 1994).

Dans le cas des substances ayant un poids moléculaire élevé, la distribution tissulaire est limitée par la diffusion de la substance au travers de la matrice cellulaire. Afin de représenter ceci, le compartiment tissulaire est sub-

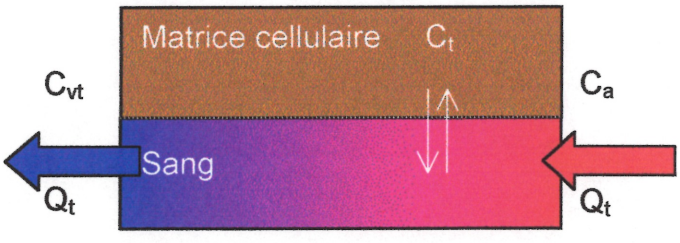

Figure 3: Compartiment d'un modèle PCBP où la distribution est limitée par la diffusion de la substance

divisé en deux sous-compartiments, l'un représentant la matrice cellulaire, et l'autre le sang (Haddad *et al.*, 1998) (Figure 3). En observant le sous-compartiment cellulaire de la Figure 3, il est possible de remarquer que le taux de ce qui entre dans le sous-compartiment dépendera de la concentration veineuse limitée par la diffusion de la substance, alors que le taux de ce qui quitte ce sous-compartiment dépendra de la concentration tissulaire, du coefficient de partage tissu:sang et de la diffusion de la substance. L'équation du bilan de masse représentant le sous-compartiment de la matrice cellulaire sera donc:

$$\frac{\delta A_{mc}}{\delta t} = D_t\left(C_{vt} - \frac{C_t}{P_t}\right) \qquad [1]$$

où $\delta A_{mc}/\delta t$ = taux de changement de la quantité de produit dans la matrice cellulaire (mg/h)

D_t = constante de diffusion tissulaire (L/h)

C_{vt} = concentration veineuse de substance dans le sous-compartiment sanguin (mg/L)

C_t = concentration tissulaire (mg/L), et

P_t = coefficient de partage tissu:sang

Par contre, pour le sous-compartiment sanguin (Figure 3), on peut remarquer que le taux de ce qui entre dans le sous-compartiment dépendra de la concentration artérielle et du débit sanguin au tissu, alors que le taux de ce qui quitte le sous-compartiment dépendra de la concentration veineuse sortant du tissu et du débit sanguin au tissu, ainsi que du taux de diffusion vers la matrice cellulaire. Dans le

sous-compartiment sanguin, le bilan de masse sera alors déterminé selon l'équation suivante:

$$\frac{\delta A_{st}}{\delta t} = Q_t C_a - Q_t C_{vt} - \frac{\delta A_{mc}}{\delta t} \qquad [2]$$

où $\delta A_{st}/\delta t$ = taux de changement de la quantité de substance dans le sang (mg/h)

Q_t = débit de sang au tissu (L/h), et

C_a = concentration artérielle de substance (mg/L)

En ce qui concerne les SOV de faible poids moléculaire, l'absorption d'une substance dans un tissu n'est pas limitée par sa diffusion, mais bien par la perfusion sanguine au tissu, c-à-d, la perfusion est lente quand elle est comparée à la diffusion (Figure 4). En observant le compartiment représenté dans la Figure 4, on peut remarquer que le taux de ce qui entre dans le compartiment dépendra de la concentration artérielle et du débit sanguin au tissu, alors que le taux de ce qui quitte le compartiment dépendra de la concentration veineuse sortant du tissu et du débit sanguin au tissu. Le bilan de masse, dans ce cas, est calculé comme suit:

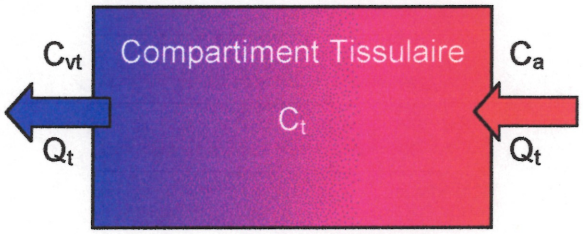

Figure 4: Compartiment d'un modèle PCBP où la distribution est limitée par la perfusion sanguine au tissu

$$\frac{\delta A_t}{\delta t} = Q_t C_a - Q_t C_{vt} \qquad [3]$$

où $\delta A_t/\delta t$ = taux de changement de la quantité de produit dans le tissu (mg/h)

L'équation 3 représente un tissu homogène et présume que la concentration veineuse tissulaire est en équilibre avec la concentration tissulaire, un équilibre qui est déterminé par le coefficient de partage tissu:sang.

Si le compartiment représente un tissu pouvant métaboliser la substance, l'équation de bilan de masse utilisée comporte un terme additionnel qui considère le taux de clairance métabolique:

$$\frac{\delta A_t}{\delta t} = Q_t C_a - Q_t C_{vt} - \frac{\delta A_{met}}{\delta t} \qquad [4]$$

où, $\delta A_{met}/\delta t$ = taux de métabolisme (mg/hr).

Le terme $\delta A_{met}/\delta t$ dans l'équation 4 peut représenter un processus métabolique d'ordre premier:

$$\frac{\delta A_{met}}{\delta t} = K_f C_{vt} V_t \qquad [5]$$

où K_f = constante métabolique de premier ordre (h^{-1}), et

V_t = volume du tissu (L).

Pour une biotransformation d'ordre premier dans le foie, l'équation suivante basée sur le concept de clairance hépatique peut être substituée à l'équation 5 (Poulin et Krishnan, 1998) :

$$\frac{\delta A_{met}}{\delta t} = Q_l \cdot E \cdot C_a \qquad [6]$$

où Q_l = débit sanguin au foie (L/h), et

E = coefficient d'extraction hépatique $[CL_{int}/(Q_l+CL_{int})]$

CL_{int} = clairance intrinsèque (V_{max}/K_m)

V_{max} = vélocité maximale du métabolisme (mg/h), et

K_m = constante d'affinité (mg/L)

Par contre, si le processus métabolique est saturable, l'équation suivante est utilisée pour représenter le taux de métabolisme:

$$\frac{\delta A_{met}}{\delta t} = \frac{V_{max} C_{vt}}{K_m + C_{vt}} \qquad [7]$$

Suite à la représentation fonctionnelle des compartiments tissulaires, les liens qui existent entre ces compartiments doivent être décrits.

DESCRIPTION DES LIENS ENTRE LES COMPARTIMENTS TISSULAIRES

Les différents compartiments tissulaires sont reliés par la circulation systémique. Le sang artériel amène la substance vers les tissus, alors que les concentrations veineuses tissulaires se regroupent pour former le sang veineux, ce qui résulte en une concentration veineuse homogène.

Certains modèles PCBP de SOV n'ont pas de compartiment distinct qui représente le sang. La concentration artérielle est plutôt représenté comme la solution, à l'équilibre, de l'équation du bilan de masse représentant le compartiment pulmonaire, où l'échange gazeux a lieu. Le taux de ce qui entre dans le compartiment pulmonaire dépend alors de la concentration inhalée et de la ventilation pulmonaire, ainsi que du débit cardiaque et de la concentraton veineuse. Par contre le taux de ce qui quitte le compartiment pulmonaire dépend de la concentration alvéolaire et de la ventilation pulmonaire, ainsi que du débit cardiaque et de la concentration artérielle (Ramsey et Andersen, 1984). Ceci est réprésenté par l'équation 8:

$$\frac{\delta A_L}{\delta t} = Q_p C_{inh} + Q_c C_v - Q_p C_{alv} - Q_c C_a \qquad [8]$$

où $\delta A_L/\delta t$ = taux de changement de la quantité de produit dans le tissu pulmonaire (mg/h)

Q_p = ventilation alvéolaire (L/h)

C_{inh} = concentration inhalée ou environnementale de la substance (mg/L)

Q_c = débit cardiaque (L/h)

C_v = concentration veineuse de la substance (mg/L), et

C_{alv} = concentration alvéolaire de la substance (mg/L).

Si l'on considère que la quantité de substance perdue de l'air inspiré est retrouvée totalement dans le sang et que le tissu pulmonaire distribue la vapeur entre l'espace alvéolaire et le sang artériel en fonction du coefficient de partage sang:air, il y a équilibre, c-à-d, $\delta A_L/\delta t = 0$. L'équation suivante peut ainsi être dérivée de l'équation 8:

$$C_a = \frac{Q_p C_{inh} + Q_c C_v}{Q_c + \frac{Q_c}{P_b}} \qquad [9]$$

où P_b = coefficient de partage sang:air

L'équation 9 reste valide tant que les trois conditions suivantes sont rencontrées: 1) il y a un équilibre rapide de la substance entre le sang et l'espace alvéolaire, 2) il n'y a aucune biotransformation significative dans le tissu pulmonaire, et 3) la capacité de stockage des poumons est négligeable. Le système sanguin veineux, quant à lui, est représenté comme la solution à l'équilibre de l'équation de bilan de masse du compartiment veineux:

$$C_v = \frac{\sum_t^n Q_t C_{vt}}{Q_c} \qquad [10]$$

La représentation fonctionnelle d'un modèle PCBP à quatre compartiments qui est utilisée fréquemment pour simuler la cinétique des SOV chez plusieurs espèces est présentée à la Figure 5.

Lorsque la description des compartiments tissulaires et des liens entre les compartiments est complétée, l'estimation des paramètres qui sont inclus dans ces équations mathématiques du modèle PCBP doit être effectuée.

1.1.2 Estimation des paramètres

Il y a trois types de paramètres (physiologiques, biochimiques, et physico-chimiques) qui servent d'intrants dans les équations de bilan de masse constituant un modèle PCBP et leurs valeurs numériques doivent être connues afin de pouvoir solutionner ces équations. Parmi les paramètres physiologiques, on note les débits sanguins, les volumes tissulaires, la ventilation pulmonaire, et le débit cardiaque. Les paramètres biochimiques incluent les constantes de métabolisme, de liaison et d'excrétion. Finalement, les paramètres physico-chimiques comprennent les coefficients de diffusion tissulaire, les constantes d'absorption, et les coefficients de partage (tissu:sang et sang:air). Parce que les modèles développés sont des modèles prédictifs, il est préférable d'obtenir la valeur de ces paramètres de façon indépendante.

1.1.2.1 Paramètres physiologiques

Les caractéristiques physiologiques d'un organisme sont représentées dans un modèle PCBP par les débits sanguins et pulmonaires, ainsi que les volumes

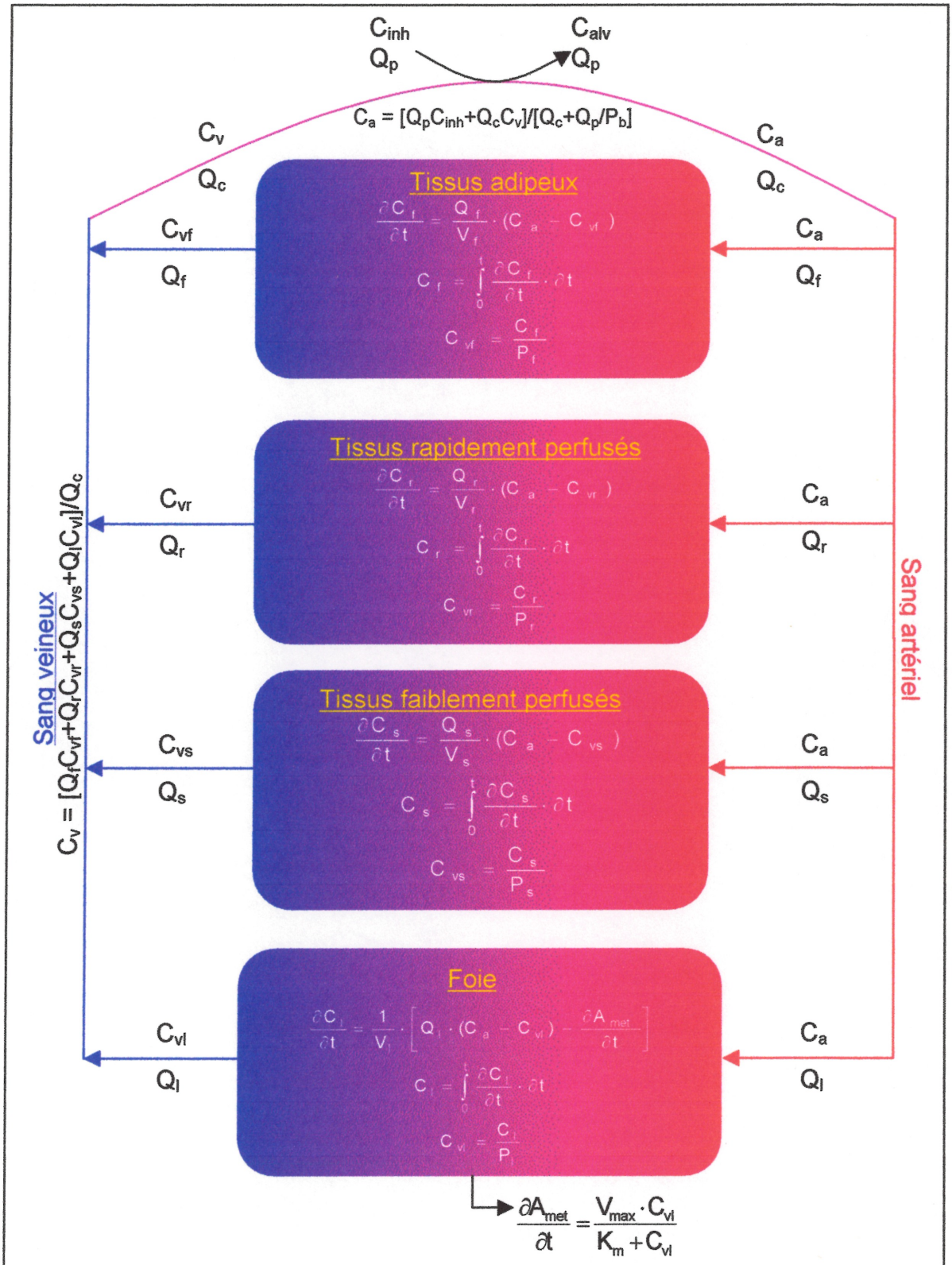

Figure 5: Représentation fonctionnelle d'un modèle PCBP pour le styrène. Adapté de Haddad *et al.* (1996).

tissulaires. Pour de nombreuses espèces (p. ex., poisson, rat, souris, ou humain)

on peut retrouver la valeur expérimentale de ces paramètres dans la littérature (Adolph, 1949; Caster *et al.*, 1956; Domench *et al.*, 1969; Arms et Travis, 1988; Ross *et al.*, 1991; Travis et Hattemer-Frey, 1991; Brown *et al.*, 1997). Lorsque les données requises ne sont pas disponibles dans la littérature, on peut les estimer à l'aide de certaines équations allométriques validées (Mordenti et Chappell, 1989; Krishnan et Andersen, 1991). Il est aussi possible de déterminer les valeurs requises expérimentalement (Mauderly, 1990; Delp *et al.*, 1991). Suite à l'estimation de la valeur des paramètres physiologiques, la valeur des paramètres biochimiques doit être déterminée.

1.1.2.2 Paramètres biochimiques

Afin de représenter adéquatement la biotransformation enzymatique ou non-enzymatique dans un tissu d'un modèle PCBP, la valeur des paramètres biochimiques doit être connue. Ces paramètres peuvent être des constantes métaboliques d'ordre zéro (en mg/h), d'ordre premier (en h^{-1}), d'ordre second (en $(mg/L)^{-1} \cdot h^{-1}$), ou saturable (V_{max} en mg/h et K_m en mg/L) (Krishnan *et al.*, 1994). Si le concept de clairance hépatique est utilisé, alors la valeur du coefficient d'extraction de la substance doit être connue (Johanson et Naslund, 1988; Yamaguchi *et al.*, 1996; Poulin et Krishnan, 1998).

La valeur des paramètres métaboliques peut être évaluée expérimentalement *in vivo* ou *in vitro*. De plus, il est aussi possible d'estimer la valeur de ces paramètres à l'aide d'équations empiriques. Les méthodes expérimentales *in vivo* utilisent la comparaison entre les valeurs de C_a ou de C_{alv} expérimentales obtenues chez l'organisme et les valeurs de C_a ou de C_{alv} telles

que simulées par un modèle PCBP. Si la valeur de tous les paramètres du modèle, sauf celles des paramètres métaboliques, est connue, alors la valeur des constantes métaboliques peut être estimée par optimisation des valeurs simulées (Pang *et al.*, 1978; Filser et Bolt, 1979; Andersen *et al.*, 1980; Gargas *et al.*, 1986; Gargas et Andersen, 1989; Gargas, 1990; Reitz *et al.*, 1990)

Les méthodes *in vitro* utilisent la vitesse de disparition d'une substance mère ou la vitesse d'apparition d'un métabolite, lorsque celle-ci est en contact avec des microsomes, fractions post-mitochondriales, suspensions cellulaires, ou cellules isolées, afin de déterminer la valeur des paramètres métaboliques (Gargas, 1991; Carfagna et Kedderis, 1992; Krishnan et Andersen, 1994).

Finalement, il existe pour plusieurs SOV et substances non-volatiles chez le rat, des équations empiriques qui peuvent être utilisées afin de déterminer la valeur de V_{max}, K_m, CL_{int}, et E. Ces équations sont basées sur la corrélation entre la valeur expérimentale de ces paramètres et certaines propriétés physico-chimiques des substances, p. ex., le coefficient de partage *n*-octanol:eau (Gargas *et al.*, 1988; Waller *et al.*, 1996; Ishizaki *et al.*, 1997).

Lorsque la valeur des paramètres biochimiques est estimée, il ne reste plus que la valeur des paramètres physico-chimiques à déterminer.

1.1.2.3 Paramètres physico-chimiques

Les modèles PCBP ont des paramètres physico-chimiques tel que le P_b et le coefficient de partage tissu:sang (P_t) qui servent d'intrant dans leurs équations. Le P_t, paramètre important de la distribution tissulaire d'une substance, est souvent calculé comme le rapport du coefficient de partage tissu:air ($P_{t:a}$) et du P_b. Dans

plusieurs cas, ces valeurs de $P_{t:a}$ et de P_b sont disponibles dans la littérature. Cependant, si elles ne le sont pas, ces valeurs peuvent être déterminées à l'aide de méthodes expérimentales *in vivo* ou *in vitro*, ou de certains algorithmes empiriques ou mécanistes.

1.1.2.3.1 Méthodes expérimentales *in vivo*

Les méthodes expérimentales pour déterminer les P_t *in vivo* se basent sur le calcul du rapport des concentrations tissulaires et sanguines au moment où celles-ci sont à l'équilibre. Deux méthodes sont couramment employées selon le type de substance étudiée. Les chambres d'inhalation sont employées dans le cas des SOV, puisque cette méthode expérimentale se prête bien aux produits volatils (Gargas et Andersen, 1989). Par contre, dans le cas des substances non-volatils, l'administration intraveineuse en infusion constante ou en bolus est employée (Chen et Gross, 1979; Lam *et al.*, 1982; Lin *et al.*, 1982; Gabrielsson *et al.*, 1984; Gallo *et al.*, 1987; Lindup, 1987). Contrairement au P_t, qui ne peut être déterminé qu'*in vivo,* la valeur du $P_{t:a}$ ou du P_b peut aussi être déterminée *in vitro*.

1.1.2.3.2 Méthodes expérimentales *in vitro*

Les $P_{t:a}$ ou P_b peuvent être déterminés *in vitro* selon plusieurs méthodes expérimentales. Dans le cas des SOV, on utilise préférablement la méthode des tubes à l'équilibre, où l'équilibre se fait entre un homogénat de tissu et d'air à une concentration d'exposition donnée. L'espace de tête du tube contenant le tissu et d'un tube de référence vide est échantillonné afin d'estimer le coefficient de partage (Sato et Nakajima, 1979; Gargas *et al.*, 1989; Kaneko *et al.*, 1994; Tardif *et al.*, 1997). Dans le cas des substances non volatiles, la méthode de dialyse à

l'équilibre (Lin *et al.*, 1982; Law *et al.*, 1991; Murphy *et al.*, 1995; Haddad *et al.*, 1998) ou la méthode d'ultrafiltration sur des composés marqués (Lin *et al.*, 1978; Jepson *et al.*, 1994), est utilisée. Ces deux méthodes sont basées sur l'équilibre entre un homogénat de tissu et une solution tampon, et les concentrations sont mesurées dans les deux phases par scintillation liquide. En plus des méthodes *in vivo* et *in vitro*, l'estimation de la valeur des P_t, $P_{t:a}$, et P_b peut se faire à l'aide d'algorithmes empiriques.

1.1.2.3.3 Algorithmes empiriques

La valeur des P_b, $P_{t:a}$ et P_t pour plusieurs SOV chez le rat et l'humain peut être estimée à l'aide d'algorithmes empiriques. Ces algorithmes sont basés sur la corrélation entre des propriétés physico-chimiques de la substance et des données expérimentales portant sur les P_b, $P_{t:a}$, ou P_t (Abraham *et al.*, 1985; Gargas *et al.*, 1988; Paterson et Mackay, 1989; Connell *et al.*, 1993). Les coefficients de partage *n*-octanol:eau ($P_{o:w}$), huile:air ($P_{o:a}$), et eau:air ($P_{w:a}$), qui représentent l'hydrophilicité ou l'hydrophobicité d'une substance, sont les propriétés avec lesquelles les meilleures corrélations sont obtenues. Les inconvénients majeurs de l'approche empirique sont que: i) ces équations ne s'appliquent qu'aux familles de substances utilisées pour les dériver, et ii) ces équations ne peuvent avancer aucune prédiction interespèce valable puisqu'elles sont dérivées à partir de valeurs déterminées expérimentalement et non sur des mécanismes biologiques. Alors, considérant ces inconvénients, certains algorithmes mécanistes ont été développés.

1.1.2.3.4 Algorithmes mécanistes

Les $P_{t:a}$ et P_b des SOV peuvent être estimées à l'aide d'algorithmes mécanistes dérivés à partir de l'équation générale suivante :

$$P_{t:a} = (P_{l:a}V_{lt}) + (P_{w:a}V_{wt}) \qquad [11]$$

où $P_{l:a}$ = coefficient de partage lipide:air

$P_{w:a}$ = coefficient de partage eau:air

V_{lt} = volume des lipides tissulaires, et

V_{wt} = volume de l'eau tissulaire

L'équation 11 se base sur un principe de Lowe et Hagler (1969) qui stipule que le partage d'une substance entre un tissu et l'air relève de sa distribution relative dans les lipides tissulaires et l'air, ainsi que l'eau tissulaire et l'air. Le terme $P_{l:a}V_{lt}$ dans l'équation 11 représente la distribution ou la solubilité d'une substance dans les lipides tissulaires, alors que le terme $P_{w:a}V_{wt}$ représente la solubilité dans l'eau tissulaire. Dans l'équation 11, la valeur du coefficient *n*-octanol:air ou le $P_{o:a}$ peut être utilisée afin d'estimer le partage entre les lipides tissulaires et l'air, alors que la réciproque de la constante d'Henry d'une substance peut être utilisée afin d'estimer le partage entre l'eau tissulaire et l'air. Même si le concept mécaniste de l'Eq. 11, c-à-d, le partage d'une substance dans un tissu dépend de sa solubilité dans les différents composants tissulaires, reste valide, l'applicabilité de cette équation à des fins prédictives est limitée car elle considère que la composition des lipides tissulaires est homogène, ce qui ne représente pas la réalité. Les lipides tissulaires sont en fait composés de lipides neutres (c-à-d, triglycérides) et

de phospholipides. De plus, les phospholipides contiennent des groupements hydrophiles (p. ex., phosphomonoester) et hydrophobes (p. ex., glycérides). Par conséquent, le $P_{o:a}$ ou le $P_{w:a}$ ne peuvent être utilisés afin d'estimer correctement la solubilité dans ce composant lipidique. L'équation 11 doit donc être modifiée afin que les différences de solubilité entre les lipides neutres et polaires soient considérées (Poulin et Krishnan, 1996b,c):

$$P_{t:a} = (P_{o:a}V_{nt}) + (0.3 \cdot P_{o:a}V_{pt}) + (0.7 \cdot P_{w:a}V_{pt}) + (P_{w:a}V_{wt}) \qquad [12]$$

où V_{nt} = fraction du volume tissulaire correspondant aux lipides neutres, et

V_{pt} = fraction du volume tissulaire correspondant aux phospholipides

Dans l'équation 12, le $P_{o:a}$ détermine le partage d'une substance dans les lipides neutres et l'air, alors que le $P_{w:a}$ détermine le partage dans l'eau tissulaire et l'air. La solubilité d'une substance dans les phospholipides est considérée comme étant la somme de 30% de la solubilité dans les lipides neutres et de 70% de la solubilité dans l'eau. Cette méthode présume que les caractéristiques physico-chimiques des phospholipides tissulaires (c-à-d, leur lipophilicité-hydrophilicité) sont semblables à celles de la lécithine (Williams et Tung 1976; Poulin et Krishnan, 1995a). Ainsi, l'équation 12 permet l'estimation de la valeur du $P_{t:a}$ à l'aide du $P_{o:a}$ et du $P_{w:a}$, qui sont propres à la substance, et de la composition tissulaire, qui est propre à l'espèce. Les valeurs de composition tissulaire et de $P_{w:a}$ sont disponibles dans la littérature (Poulin et Krishan, 1995a, 1996b,c). Par contre, il est parfois plus facile d'obtenir la valeur du $P_{o:w}$ d'une substance plutôt que sa valeur de $P_{o:a}$. Afin

d'utiliser la valeur du $P_{o:w}$, l'équation 12 peut être réarrangée comme suit (Poulin et Krishnan,1996c):

$$P_{t:a} = [P_{o:w}P_{w:a}(V_{nt} + 0.3V_{pt})] + [P_{w:a}(V_{wt} + 0.7V_{pt})] \quad [13]$$

Les équations 12 et 13 ont été validées en comparant les valeurs prédites aux valeurs expérimentales de $P_{t:a}$ prises de la littérature. Ces équations peuvent prédire la valeur du $P_{t:a}$ de plusieurs tissus (hépatique, musculaire et adipeux) pour plusieurs SOV chez le rat et l'humain (Poulin et Krishnan, 1995a,b, 1996b,c). En général, la solubilité dans le *n*-octanol ou l'huile végétale est adéquate afin de prédire la solubilité dans les lipides neutres biotiques. Cependant, pour les SOV relativement hydrophiles de la famille des alcools, esters d'acétate, et cétones, le $P_{t:a}$ prédit à l'aide de la solubilité dans le *n*-octanol est différent de celui prédit à l'aide de la solubilité dans l'huile. Cette différence entre les valeurs prédites et expérimentales n'est pas observée dans le cas de SOV plus lipophiles (p. ex., alcanes, haloalcanes, et aromatiques) et la structure moléculaire même du *n*-octanol en est possiblement responsable (Poulin et Krishnan, 1996c). En effet, le *n*-octanol, contrairement aux lipides neutres tissulaires et à l'huile végétale, possède une fonction alcool, ce qui faciliterait la solubilisation de substances hydrophiles (Poulin et Krishnan, 1995b, 1996c). Ainsi, lors de l'estimation de la valeur du $P_{t:a}$ de SOV hydrophiles, la solubilité de la substance dans les lipides biotiques devrait correspondre à la solubilité dans l'huile végétale (Poulin et Krishnan, 1995b, 1996c). Par contre, lors de l'estimation de la valeur du $P_{t:a}$ de

SOV lipophiles, la solubilité dans les lipides biotiques peut correspondre à la solubilité dans le *n*-octanol ou dans l'huile végétale (Poulin et Krishnan, 1996c).

La valeur du P_b d'un SOV peut être estimée à l'aide de l'équation suivante qui est basée sur l'équation 13:

$$P_b = [P_{o:w}P_{w:a}(V_{nb} + 0.3V_{pb})] + [P_{w:a}(V_{wb} + 0.7V_{pb})] \quad [14]$$

où V_{nb} = fraction du volume de sang correspondant aux lipides neutres

V_{pb} = fraction du volume de sang correspondant aux phospholipides, et

V_{wb} = fraction du volume de sang correspondant à l'eau

Chez l'humain, cette équation permet la prédiction de la valeur du P_b de plusieurs SOV (Poulin et Krishnan, 1995a). Les données sur la composition du sang humain, le $P_{o:w}$, et le $P_{w:a}$, nécessaires à la prédiction, sont toutes disponibles dans la littérature (Poulin et Krishnan, 1995a). Chez le rat, l'équation 14 peut estimer adéquatement la valeur du P_b de SOV hydrophiles (p. ex., alcools, cétones, et aromatiques), mais la valeur du P_b de SOV lipophiles (p. ex., alkanes, haloalkanes, et aromatiques) est sous-estimée de 60 à 80% (Poulin et Krishnan, 1995b, 1996a,c). Cette divergence entre les valeurs prédites et expérimentales peut être expliquée par la présence d'un phénomène de liaison aux composants protéiniques sanguins du rat qui est cumulé à la solubilité dans les lipides et l'eau du sang (Poulin et Krishnan, 1996a). Alors que la solubilité sera déterminée par la composition du sang en eau, lipides neutres, et phospholipides, ainsi que par les caractéristiques physico-chimiques de la substance, la liaison aux protéines du sang sera déterminée par la concentration de protéines plasmatiques et/ou

d'hémoglobine, et par l'affinité de la substance pour ces protéines (Poulin et Krishnan, 1996a). Puisque l'équation 14 ne considère que la solubilité d'une substance dans les lipides et l'eau du sang et non la liaison potentielle aux protéines du sang de rat, elle sous-estime la valeur du P_b pour plusieurs SOV lipophiles. Poulin et Krishnan (1996a) ont d'ailleurs utilisé cette divergence entre les valeurs prédites et expérimentales chez le rat afin d'estimer de façon indirecte la valeur de la constante d'association (K_a) à l'hémoglobine pour plusieurs SOV lipophiles. Une étude expérimentale systématique pouvant déterminer la valeur de K_a et du nombre de sites de liaison (n) pour la liaison protéinique de SOV n'a jamais été entreprise auparavant.

Plusieurs études suggèrent que l'hémoglobine pourrait être la protéine sanguine responsable de la discordance observée entre les valeurs estimées et expérimentales chez le rat (Featherstone et Schoenborn, 1964; Lam *et al.*, 1990; Poulin et Krishnan, 1996a), mais ceci n'a jamais été démontré expérimentalement. Ainsi, des études plus approfondies concernant la liaison de SOV lipophiles au protéines du sang est nécessaire afin que le P_b du rat soit correctement représenté en modélisation PCBP.

1.2 Problématique

Présentement, la liaison potentielle des SOV aux protéines du sang de rat n'est pas considérée lors de la détermination du P_b. De plus, une seule concentration d'exposition est utilisée *in vitro* afin de déterminer expérimentalement la valeur du P_b et celui-ci est considéré comme un intrant invariable dans la modélisation PCBP (Sato et Nakajima, 1979; Gargas *et al.*,

1989; Krishnan et Andersen, 1994; Poulin et Krishnan, 1996a). La présence de la liaison protéinique entrainerait certainement une variation de la valeur du P_b en fonction de la concentration d'exposition à cause de la saturation des sites de liaison. Cette variation de la valeur du P_b pourrait avoir un impact majeur sur les extrapolations animal-humain et les extrapolations haute dose-faible dose qui utilisent les modèles PCBP comme approche en analyse du risque toxicologique puisque: i) les données toxicologiques chez le rat sont souvent recueillies à des concentrations d'expositions élevées, et ii) il semble avoir absence de liaison aux protéines sanguines chez l'humain (Poulin et Krishnan, 1995a).

Bien que quelques études aient utilisé la liaison des SOV aux protéines du sang afin d'expliquer certains résultats (Kumarathasan *et al.*, 1998; Lam *et al.*, 1990; Medinsky *et al.*, 1988; Poulin et Krishnan, 1996a; Travis et Bowers, 1989), aucune étude portant sur: i) l'identification des caractéristiques des SOV se liant aux protéines, ii) l'identification de la protéine spécifiquement responsable de la liaison dans le sang, iii) la détermination expérimentale de la valeur de K_a et de n, et iv) la base scientifique et la nature de variation du P_b en fonction de la concentration d'exposition, n'a encore été effectuée. Ces informations sont esssentielles pour mieux comprendre la liaison protéinique des SOV lipophiles dans le sang de rat et son rôle lors des extrapolations utilisant la modélisation PCBP.

1.3 Objectifs

Ce projet de recherche visait à évaluer la liaison des SOV aux protéines sanguines chez le rat dans des conditions *in vitro*. Les objectifs spécifiques étaient:

i) d'identifier, suite à une revue de la littérature, les caractéristiques communes aux SOV pouvant être potentiellement impliquées dans la liaison aux protéines du sang, ii) de développer un algorithme pour estimer le P_b en tenant compte de la liaison protéinique qui soit utilisable en modélisation PCBP, iii) de vérifier expérimentalement la présence de la liaison, telle que prédite par les caractéristiques précédemment identifiées, de certain SOV aux protéines sanguines du rat, iv) d'étudier l'implication de l'hémoglobine (Hb) dans cette liaison, v) de déterminer l'impact de la concentration d'exposition sur le P_b, et, vi) de déterminer la valeur de la constante d'affinité (K_a) et le nombre de sites de liaisons (n) des protéines impliquées.

1.4 Organisation

Les objectifs du présent projet de recherche ont été réalisés en trois étapes. Premièrement, les SOV identifiés dans la littérature comme étant théoriquement liés aux protéines du sang chez le rat ont été analysés afin de discerner certaines caractéristiques communes (Article I). Ainsi, des caractéristiques portant sur la dimension et la composition moléculaire des SOV ont été identifiées comme étant des éléments importants de la liaison aux protéines. De plus, dans l'article I, un algorithme qui peut être utilisé en modélisation PCBP afin d'estimer le P_b a été développé.

Ensuite, en se basant sur ces caractéristiques communes, la présence ou l'absence de liaison a été prédite pour certains SOV. La présence de cette liaison a ensuite vérifiée expérimentalement (Article II). De plus, la contribution de l'Hb à la valeur du P_b a été évaluée.

Finalement, l'effet de la concentration d'exposition sur le P_b, ainsi que la valeur de K_a et de n ont été déterminés pour certains SOV par la méthode des tubes à l'équilibre (Article III).

CHAPITRE DEUXIÈME :

2 – ARTICLE I

Dans l'article suivant, la recherche sur les déterminants moléculaires de la liaison aux protéines du sang de rat, ainsi que le développement d'un algorithme qui considère cette liaison dans l'estimation du P_b a été effectuée par M. Béliveau. En plus, M. Béliveau a participé a la rédaction de l'article.

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Mechanistic Animal-Replacement Approaches for Predicting Pharmacokinetics of Organic Chemicals

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Introduction

The prediction of the pharmacokinetic behavior of chemicals prior to experimentation in animals and humans is a major challenge. Physiologically-based pharmacokinetic (PBPK) models represent a useful framework for simulating the tissue and blood concentration profiles of chemicals in intact animals and humans for various exposure scenarios, routes, and dose levels[1]. The PBPK models refer to mathematical descriptions of absorption, distribution, metabolism and elimination of chemicals in biota based on proven/hypothetical interrelationships among certain mechanistic determinants[2,3]. The basic mechanistic determinants included in PBPK models are the physiological characteristics (e.g., cardiac output, alveolar ventilation rate, tissue volumes, tissue blood flow rates), biochemical parameters (e.g., maximal velocity and affinity for metabolism), and physicochemical parameters (e.g., blood:air partition coefficients, tissue:blood partition coefficients, dermal permeability coefficients) which together determine the pharmacokinetic behavior of chemicals in biota (Table 1).

For constructing PBPK models, numerical values of the physiological, physico-chemical and biochemical parameters need to be known. The values of physiological parameters for several species are available in the biomedical literature[1]. However, the physico-chemical and biochemical parameters need to be determined *in vivo* or *in vitro* for each chemical [1,4-6]. Alternatively, these parameters can be predicted using animal-replacement approaches. The most commonly used approach in this regard involves the development of empirical

equations relating the values of these parameters (e.g. partition coefficients[7-35], dermal permeability coefficient[4, 36-53] and biochemical rate constants [8, 54-64]) to some other basic chemical-specific parameter such as the log *n*-octanol:water partition coefficient or molecular connectivity indices. Following the incorporation of this kind of equations within PBPK models, the numerical values of partition coefficients and biochemical parameters for members of homologous series of chemicals have been obtained and, in turn, used to simulate their pharmacokinetics in animals[65-66]. This approach is essentially a "fitting" exercise that requires collection of extensive experimental data and its usefulness is limited to the class of compounds for which the data are initially collected. It would be more useful to develop animal replacement approaches that can provide the numerical values of PBPK model parameters regardless of chemical class. Such approaches should essentially be based on an understanding of the mechanistic determinants of the input parameters required for PBPK modeling.

This chapter presents mechanistic animal-replacement approaches for predicting certain chemical-specific input parameters of PBPK models, namely partition coefficients and biochemical parameters, and their application to predict *in vivo* pharmacokinetics of organic chemicals.

Mechanistic Animal-replacement Approaches for Predicting Partition Coefficients

The partition coefficients refer to the ratio of chemical concentration in two phases (e.g., tissue and air, blood and air, tissue and blood) at equilibrium. In general terms, the partitioning of a chemical between two matrices can be predicted if its solubility and binding in each of the two matrices can be estimated with reasonable accuracy. Using this basic premise, mechanistic animal-replacement approaches for predicting tissue:air, blood:air and tissue:blood partition coefficients (PCs) have been developed.

Tissue:air PCs

Tissue:air PCs of low-molecular weight volatile organic chemicals (VOCs), for which macromolecular binding is negligible, can be calculated based on the following general equation[12, 67-68]:

$$P_{t:a} = (P_{l:a} * V_{lt}) + (P_{w:a} * V_{wt}) \qquad [1]$$

where $P_{l:a}$ = lipid:air partition coefficient, $P_{w:a}$ = water:air partition coefficient, V = volume of tissue components, namely, lipids (lt) and water (wt).

In the above equation, $P_{l:a}*V_{lt}$ represents the partitioning of a chemical between tissue lipids and air, and $P_{w:a}*V_{wt}$ represents the partitioning between tissue water and air. In this context, $P_{o:a}$ (oil:air or *n*-octanol:air partition coefficients) of chemicals have been used as a surrogate for $P_{l:a}$, and $P_{w:a}$, the reciprocal of Henry's law constant, has been used as a surrogate of chemical

partitioning between tissue water and air [12, 67-68]. Tissue lipids, in fact, represent both neutral (e.g., triglycerides) and polar lipids (e.g., phospholipids). Therefore, the partitioning of a chemical into neutral and polar lipids needs to be considered separately. The physicochemical properties of phospholipids are dependent on the presence of hydrophobic (e.g., glyceride) and hydrophilic (e.g., phosphomonoester) groups. Therefore, using *n*-octanol or water solubility information alone to predict chemical solubility in tissue phospholipids may not be appropriate. Poulin and Krishnan[69-70] proposed the following equation to predict $P_{t:a}$ of VOCs, considering separately the partitioning into neutral and polar lipids:

$$P_{t:a} = (P_{o:a}V_{nt}) + (0.3P_{o:a}V_{pt}) + (0.7P_{w:a}V_{pt}) + (P_{w:a}V_{wt}) \quad [2]$$

where V_{nt} = volume fraction of neutral lipids, and V_{pt} = volume fraction of phospholipids in tissue t.

In the above Eqn., the partitioning of a chemical between tissue neutral lipids and air corresponds directly to $P_{o:a}$, whereas the partitioning between tissue water and air is set equal to $P_{w:a}$. However, the partitioning of a chemical between polar lipids (i.e., phospholipids) and air is calculated as a fractional additive function of the partitioning into neutral lipids ($0.3*P_{o:a}$) and water ($0.7*P_{w:a}$). The latter method of approximation of chemical partitioning into tissue phospholipids is based on the assumption that the lipophilicity-hydrophilicity characteristic of tissue phospholipids is similar to that of commercial lecithin[71-72]. According to Eqn. 2, $P_{t:a}$ can be calculated with knowledge of tissue composition data, $P_{o:a}$ and $P_{w:a}$. Species-specific data on tissue composition are available in the literature (Table 2).

The $P_{o:a}$ and $P_{w:a}$ values for a number of chemicals can be obtained from the literature[7, 10, 12, 16, 73]. However, it is easier to obtain *n*-octanol:water or oil:water ($P_{o:w}$) values than $P_{o:a}$ values from the literature. To use the $P_{o:w}$ values rather than $P_{o:a}$ which are not available frequently, Eqn. 2 can be rewritten as follows[70]:

$$P_{t:a} = [P_{o:w} P_{w:a} (V_{nt} + 0.3V_{pt})] + [P_{w:a}(V_{wt} + 0.7V_{pt})] \quad [3]$$

Eqns. 2 & 3 have been used to predict rat and human $P_{t:a}$ (liver, muscle, fat) of several VOCs[69-70, 74-76]. In general, the $P_{t:a}$ values predicted using the animal-replacement approaches were within a factor of two of the experimental data (Figure 1).

$P_{o:w}$ in Eqn. 3 refers to *n*-octanol:water PCs or vegetable oil:water PCs of chemicals. In general, *n*-octanol and vegetable oil have been considered as useful surrogates of biotic neutral lipids[12, 77-78]. However, systematic differences between *n*-octanol:water PCs and oil:water PCs are observed for relatively hydrophilic VOCs (i.e., alcohols, acetate esters, ketones), but this is not the case for relatively lipophilic VOCs (i.e., alkanes, haloalkanes, aromatics)[69-70, 77]. *n*-Octanol, being an alcohol, would appear to solubilize other hydrophilic organics to a greater extent than do biotic neutral lipids. Based on the hydrophilicity-lipophilicity characteristics and the fatty acid composition, vegetable oil has been suggested to be an acceptable alternative to *n*-octanol as a surrogate of biotic neutral lipids, especially for hydrophilic organics[77]. Then, to predict $P_{t:a}$ of hydrophilic VOCs, especially for fatty tissues, the $P_{o:w}$ in Eqn. 3 should represent vegetable oil:water PCs. However, in the case of relatively lipophilic VOCs (log $P_{o:w}$ > 1.25), there is no

difference as to whether *n*-octanol:water PCs or vegetable oil:water PCs are used as the biotic lipid surrogates[69-70, 77].

Tables 3 – 5 compare the predictions of adipose tissue:air, liver:air and muscle:air PCs of several chemicals obtained using the mechanistic animal-replacement approach presented above (Eqn. 3), with experimental data collected previously using rat tissues. On average, the predicted rat adipose tissue:air PCs varied by a factor of 1.16 (SD = 0.65, n=27) from the experimental values. The average ratios of the predicted/experimental values were 0.97 (SD = 0.44) and 0.74 (SD = 0.47), respectively, for liver:air and muscle:air PCs. The rat tissue:air PCs have, in general, been used for not only developing rat PBPK models but also human PBPK models, with the assumption that the tissue:air PCs are species-invariant. However, some previous efforts indicated that the liver:air and adipose tissue:air PCs are comparable between species (rat vs. human), whereas the muscle:air PCs are somewhat different[70, 76]. In this context, it might be useful to undertake a systematic comparison of rat and human muscle:air PCs of VOCs. With the availability of mechanism-based algorithms, it becomes easier to evaluate the impact of species differences in tissue composition on the PCs and pharmacokinetics of chemicals.

Blood:air PCs

The blood:air PC ($P_{b:a}$) is an important input parameter for PBPK modeling of VOCs. This parameter has been calculated in the past using modified forms of

Eqn. 1 that require data on the lipid, water and/or protein contents of whole blood[11-12, 68, 78-80]. Some previous studies[68, 78-80] lumped together the neutral and polar lipid components of blood during the calculations, and only performed limited validation. Other investigators[11-12], using a semi-empirical approach to predict $P_{b:a}$, presented a systematic validation of their results. Based on Eqn. 3, Poulin and Krishnan[70, 81] proposed the following mechanistic animal-alternative approach for predicting $P_{b:a}$ of VOCs that do not bind significantly to blood proteins:

$$P_{b:a} = [P_{o:w} P_{w:a} (V_{nb} + 0.3V_{pb})] + [P_{w:a}(V_{wb} + 0.7V_{pb})] \quad [4]$$

where V = volume fraction of blood components and the subscripts nb, pb and wb refer to neutral lipids, phospholipids, and water.

Using Eqn. 4, $P_{b:a}$ of VOCs can be calculated with the knowledge of blood composition data, $P_{o:w}$ and $P_{w:a}$. The data on lipid and water levels in rat and human blood are available in the literature[70,81] and so are the numerical values of $P_{o:w}$ and $P_{w:a}$ at 37°C for several VOCs[7, 10, 12, 16, 17, 73, 78-80, 82]. The predictions of $P_{b:a}$ obtained using Eqn. 4 are adequate for relatively hydrophilic organics (i.e., alcohols, ketones, acetate esters), but it is not the case for relatively lipophilic VOCs (i.e., alkanes, haloalkanes and aromatic hydrocarbons)[70, 81](Figure 2). For most alkanes, haloalkanes and aromatic hydrocarbons, the rat $P_{b:a}$ values obtained using Eqn. 4 were lower (60 – 80 %) than the experimental data [70, 81]. The $P_{b:a}$ of a chemical is a composite number that potentially represents two processes occurring in the blood, namely, solubility and binding. Whereas the

solubility is likely to be determined by the neutral lipid, phospholipid and water components of blood, the binding would appear to be associated with plasma proteins and/or hemoglobin. The underpredictions of $P_{b:a}$ of lipophilic VOCs by Eqn. 4 can be explained by the lack of the consideration of potential binding of these chemicals to blood proteins[81, 87, 88]. Larson *et al.*[79] measured under *in vitro* conditions the hemoglobin:air PCs of halothane, and considered these data during calculations of human $P_{b:a}$. Poulin and Krishnan[81] derived the binding association constants ($1930 \pm 819\ M^{-1}$) for alkanes, haloalkanes and aromatic hydrocarbons in rat blood from *in vitro* experimental data.

Whereas a large discrepancy exists between the experimental values and solubility-based predictions of $P_{b:a}$ for relatively lipophilic VOCs, such differences are not apparent in the case of alcohols, ketones and acetate esters[81]. The underestimation of the $P_{b:a}$ of lipophilic VOCs can be explained by possible binding or localization of these chemicals in the hydrophobic pockets of hemoglobin[81, 87, 88]. In order to uncover the determinants of such a process, the lipophilicity characteristics and molecular volumes of several chemicals that have been hypothesized to and not to bind to rat hemoglobin (Table 6) were examined. Figure 3 shows a scatterplot of log $P_{o:w}$ values against geometric volumes of chemicals. The chemicals segregate into two distinct groups, the more leftward group comprising of oxygen-containing chemicals (i.e., ketones, alcohols, acetate esters), and the more rightward group comprising of the oxygen-lacking lipophilic chemicals. Note that VOCs hypothesized to bind to rat hemoglobin are all located within the range limited by a log $P_{o:w}$ value > 1, and a molecular volume < 300 cubic Angstroms. Three outliers can be identified in this scheme (methyl *iso*butyl

ketone, *n*-propyl acetate ester and *iso*propyl acetate ester), all of which possess a significant hydrophilic surface (18, 38 and 29%, respectively). The presence of hydrophilic surface is due to the oxygen atoms contained in these molecules, which can pave way for hydrogen bond formation thereby impeding the localization of these substances within the hydrophobic hole in hemoglobin.

It would appear that molecules with (i) volume greater than 300 cubic Angstroms, (ii) a log $P_{o:w}$ value < 1, or (iii) an oxygen atom in their structure do not bind significantly to hemoglobin, such that solubility-based predictions of $P_{b:a}$ are adequate. This is supported by the fact that for several alcohols, acetate esters and ketones, rat $P_{b:a}$ can be adequately predicted only by considering the solubility in blood lipids and water[81]. This hypothetical binding phenomenon for lipophilic VOCs appears to be less marked in human blood[70]. For relatively hydrophilic VOCs, human $P_{b:a}$ can be adequately predicted using blood composition data (Table 7), $P_{o:w}$ and $P_{w:a}$ values in Eqn. 4, i.e., without the consideration of hemoglobin binding (Table 8). Additional data presented in Table 9 confirm that for some of these chemicals, the solubility-based algorithm does certainly not underestimate the components of $P_{b:a}$ (i.e., erythrocyte:air and plasma:air PCs). Since the predicted PC values are only greater and not less than the experimental values, hemoglobin binding of hydrophilic VOCs in humans is not likely to be important, as in the rat[81].

In cases where hemoglobin binding is important, the apparent $P_{b:a}$ can be calculated as follows:

$$P_{b:a,(app)} = \frac{C_{a(total)}}{C_{air}} \qquad [5]$$

where C_a = arterial blood concentration and C_{air} = atmospheric concentration. Considering the components of $C_{a(total)}$, Eqn. 5 can be rewritten as follows:

$$P_{b:a(app)} = \frac{C_{a,free}}{C_{air}} + \frac{C_{a,bound}}{C_{air}} \quad [6]$$

Since $C_{a,\,bound} = C_{a,\,free}K_aC_p/(1+K_aC_{a,\,free})$,

$$P_{b:a(app)} = \frac{C_{a,free}}{C_{air}} + \left[\frac{C_{a,free} * K_a * C_p}{C_{air} * \left(1 + K_a * C_{a,free}\right)}\right] \quad [7]$$

where K_a = binding association constant and C_p = concentration of binding proteins.

Eqn. 7 can be rewritten as,

$$P_{b:a(app)} = \frac{C_{a,free}}{C_{air}} * \left[1 + \frac{K_a * C_p}{1 + K_a * C_{a,free}}\right] \quad [8]$$

The first term of Eqn. 8 corresponds to solubility-based $P_{b:a}$ predicted using Eqn.4. Therefore, replacing $C_{a,\,free}/C_{air}$ with $P_{b:a}$, Eqn 8 reads:

$$P_{b:a(app)} = P_{b:a(pred)} * \left[1 + \frac{K_a * C_p}{1 + K_a * C_{a,free}}\right] \quad [9]$$

This equation can be incorporated within PBPK models to calculate the $P_{b,app}$ as a function of time and exposure concentration. Here, the only additional, chemical specific parameter that is required relates to K_a. At the present time, there does not exist a validated animal-replacement algorithm for predicting association constants for blood protein binding of organic chemicals. However, based on the analysis presented by Poulin and Krishnan[81], it would appear that the average K_a

value for rat hemoglobin binding is 1930 $M^{-1}$for several VOCs (i.e., chemicals with a molecular volume of < 300 cubic Angstroms, log $P_{o:w}$ >1, and lacking oxygen in the molecule). This information may be used, at the present time, to provide a first-cut estimate of K_a and $P_{b:a(app)}$ for purposes of PBPK modeling of VOCs in the absence of experimental data.

Tissue:blood partition coefficients

Tissue:blood PCs ($P_{t:b}$) of VOCs are calculated by dividing the tissue:air PCs with the blood:air PC. For organic chemicals, for which macromolecular binding in tissues and blood is negligible, $P_{t:b}$ can be estimated from $P_{o:w}$ using the following general Eqn.[32, 89-92]:

$$P_{t:b} = \frac{(P_{o:w} V_{lt}) + V_{wt}}{(P_{o:w} V_{lb}) + V_{wb}} \qquad [10]$$

Some authors considered V_{lt} and V_{lb} in Eqn. [10] to represent neutral or total lipid content of tissues and blood, and tested the validity of their approach in a limited manner[32, 89, 92] . Also, they assumed that tissues and blood are constituted exclusively of neutral lipids and water (i.e., $V_{lt} + V_{wt} = 1$) during calculations of $P_{t:b}$ with Eqn. 10[32, 89-90, 92]. This is unrealistic, considering the fact that some tissues contain as much as 15% protein[84, 86]. Accounting for differential solubility of chemicals in neutral lipids, phospholipids and water, and the actual fractional volumes of these components in tissues and blood, Poulin and Krishnan[70, 72, 77] proposed the following algorithm for predicting $P_{t:b}$ of VOCs:

$$P_{t:b} = \frac{P_{o:w}(V_{nt} + 0.3V_{pt}) + (V_{wt} + 0.7V_{pt})}{P_{o:w}(V_{nb} + 0.3V_{pb}) + (V_{wb} + 0.7V_{pb})} \quad [11]$$

The predictions of $P_{t:b}$ obtained using Eqn. 11 are identical to the ratio of the predictions of $P_{t:a}$ and $P_{b:a}$ obtained with Eqns 3 and 4.

The mechanistically-based animal-replacement algorithms presented in the preceding paragraphs are useful for predicting PCs of low-molecular weight VOCs required for PBPK modeling. These approaches are also applicable for prediction of $P_{t:b}$s of non-volatile organic chemicals, which are neither ionized to a significant extent nor bound in the parent chemical form to biological macromolecules[32]. In case where ionization is important, the fraction ionized in the aqueous phase (as a function of the pH of biological fluids) should be taken into account. The consideration of binding to calculate apparent $P_{t:b}$ can be done with knowledge of K_a and C_p. Even though the $P_{t:a}$, $P_{b:a}$ and $P_{t:b}$ of organic chemicals can be predicted using animal-alternative approaches, their pharmacokinetic profiles can be predicted using PBPK framework only if the metabolic rate constants are known.

Mechanistic Animal-replacement Approaches for Predicting Biochemical Parameters

The major constraint for developing PBPK models prior to the conduct of *in vitro* or *in vivo* studies in animals and humans relates to reliable prediction of the

pathways and rates of biochemical processes in the intact organism. Softwares and expert systems are available for predicting the plausible metabolic pathways and potential metabolites of chemicals[93-95]. However, there does not exist a validated methodology for providing quantitative predictions of the chemical-specific metabolic constants (i.e., maximal velocity, V_{max} ; Michaelis affinity constant, K_m).

In PBPK models, the rate of hepatic metabolism (RAM) for first order conditions is often described as follows:

$$RAM = CL_{int} \times C_{vl} \qquad [12]$$

where $CL_{int} = V_{max}/K_m$ (L/hr) and C_{vl} = chemical concentration in venous blood leaving liver (mg/L).

To solve the above Eqn., numerical values of V_{max} and K_m, or that of CL_{int} are required. An alternative approach of simulating RAM in PBPK models would involve the use of an equation based on the classical hepatic clearance concept[96], that recognizes the role of blood flow rate in limiting hepatic metabolism. Accordingly, RAM can be calculated with the following alternative forms:

$$RAM = CL_h \times C_a, \text{ or } Q_l \times E \times C_a \qquad [13]$$

where CL_h = hepatic clearance (L/hr), Q_l = blood flow rate to the metabolizing organ (L/hr), and E = extraction ratio [i.e., organ clearance (L/hr)/organ blood flow rate (L/hr)].

Using Eqn. 13, particularly the form Q_l*E*C_a instead of the conventional form (i.e., V_{max}/K_m * C_{vl}) to describe first order metabolism in PBPK models is a starting point towards the exploration of animal-replacement approaches for simulating pharmacokinetics of chemicals. Before using Q_l*E*C_a to simulate hepatic metabolism in PBPK models, it is essential to verify that this form and Michaelis-Menten equation based form are mathematically equivalent. Recently Poulin and Krishnan[75] have demonstrated the conceptual and mathematical equivalence of these two forms of equations. The determination of the equivalence was based on the fact that (i) C_{vl} equals C_a (1-E), and (ii) V_{max}/K_m = Q_l*E/(1-E). The use of Eqn. 13 permits to simulate the kinetics of chemicals with no knowledge of the actual numerical values of V_{max} and K_m, if the value of E can be specified. The E value, which represents the chemical-specific hepatic extraction ratio, or the magnitude of CL_h to Q_l cannot be obtained *a priori* at the present time without laboratory experimentation. Actually, V_{max} and K_m should be known in order to calculate the E values. However, it is common knowledge that for highly extracted chemicals, CL_h approximates Q_l, and for poorly extracted chemicals $CL_h \approx 0$. In other words, E (= CL_h/ Q_l) of chemicals range between 0 and 1. Therefore, by specifying E = 0 or 1 in Eqn. 13 within PBPK models, predictions of the plausible envelope of tissue and blood concentration profiles can be generated for any inhaled VOC[75]. This methodology, which considers complete or zero hepatic extraction, can provide an

envelope within which all experimental data should fall. Figures 4 & 5 present the predicted range of blood concentration profiles and their comparison with experimental data following inhalation exposure to 80 ppm of styrene in the rat and 100 ppm of dichloromethane in humans[97-98]. For generating these simulations, the E value in the PBPK model was initially set equal to 0 and then to 1, and the PCs were predicted based on Eqns. 3, 4 and 9, respectively, in the human and rat PBPK models. This animal-replacement modeling approach is useful for VOCs with low $P_{b:a}$, since the ratio of the area-under the blood concentration vs time curves (AUC) obtained for conditions of E=0 and E=1 increases with increasing $P_{b:a}$ [75].

Even though $AUC_{E=0/E=1}$ might be enormous in some cases, it only reflects the fact that this could actually happen in a population where the E value varies substantially, i.e., between 0 to 1[99]. The use of the range of E values not only accounts for the inter-individual differences in metabolism rates, but also for the possible effects of co-exposures. Metabolic interactions during co-exposures result in an increase or decrease of the extraction ratios; regardless of the mechanism, nature and number of the interacting chemicals, the E value of a chemical will not exceed the range of 0 and 1. This animal-replacement approach can therefore be used to provide a first-cut estimation of the range of tissue doses and blood concentrations of chemicals that might result from specific exposure conditions. Further research to develop mechanism-based approaches for predicting animal-specific values and population distribution of the values of V_{max}

and K_m is required. In this context, approaches based on thermodynamic principles[100-103] may be useful.

As detailed in this chapter, some progress has been made to develop mechanistic animal-replacement approaches for predicting pharmacokinetics of chemicals prior to animal or human experimentation. The applicability of these approaches has largely been verified with inhaled VOCs. Even though these approaches are conceptually applicable to nonvolatile organics as well, it becomes more challenging to predict the other PBPK model parameters required for modeling the kinetics of these chemicals (i.e. tissue diffusion coefficients, tissue binding association constants, oral absorption rates, and dermal permeability coefficients). As our level of understanding of the mechanistic determinants of each of these parameters improves, animal-replacement approaches to provide *a priori* predictions of these parameters can be developed. If similar strategies are explored for developing pharmacodynamics models as well, then animal-replacement approaches of modeling the toxicity of chemicals may become a realistic goal in the long run.

REFERENCES

1. K. Krishnan and M.E. Andersen, Physiologically-based pharmacokinetic modeling in toxicology, In Principles and methods in toxicology, ed. W. Hayes, 3rd edn, pp. 149-188. Raven Press, New York (1994).

2. R. Jain, L. E. Gerlowski, J.M. Weissbrod, J. Wang, and R.N. Pierson, Kinetics of uptake, distribution and excretion of zinc in rats, Ann. Biomed. Eng. 9, 347-361 (1982).

3. K.B. Bischoff, R.L. Dedrick, D.S. Zakharo, and J.A. Longstreth, Methotrexate pharmacokinetics, J. Pharm. Sci. 60, 1128-1133 (1971).

4. United States Environmental Protection Agency (USEPA), Dermal exposure assessment: principles and applications, Document EPA/600/8-91/011B, Office of health and environmental assessment (1992).

5. F.P. Guengerich, *In vitro* techniques for studying metabolism. J. Pharmacokinet. Biopharm. 24, 521-533 (1996).

6. D. Howes, R.H. Guy, J. Hadgraft, J. Heylings, U. Hoeck, F. Kemper, H. Maibach, J.P. Marty, H. Merk, J. Para, D. Rekkas, I. Rondelli, H. Schaefer, U. Tauber and N. Verbiese, Methods for assessing percutaneous absorption. ATLA 24, 81-106 (1996).

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

8. M.L. Gargas, P.G. Seybold and M.E. Andersen, Modeling the tissues solubilities and metabolic rate constants (V_{max}) of halogenated methanes, ethanes, and ethylenes. Toxicol. Lett. 43, 235-256 (1988).

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

10. T. Kaneko, P.Y. Wang and A. Sato, Partition coefficients of some acetate esters and alcohols in water, blood, olive oil, and rat tissues. Occup. Environ. Med. 54, 68-72 (1994).

11. Des W. Connell, R.D. Braddock and S.V. Mani, Prediction of the partition coefficient of lipophilic compounds in the air-mammal tissues systems. Sci. Total Environ. Suppl., 1383-1396 (1993).

12. S. Paterson and D. Mackay, Correlation of tissue, blood, and air partition coefficients of volatile organic chemicals. Br. J. Ind. Med. 46, 321-328 (1989).

13. E.I. Eger and C.P. Larson, Jr., Anaesthetic solubility in blood and tissues; values and significance. Br. J. Anaesth. 36, 140-149 (1964).

14. F. Lombardo, J.F. Blake and W. Curatolo, Computation of brain-blood partitioning of organic solutes via free energy calculations. J. Med. Chem. 39, 4750-4755 (1996).

15. M.H. Abraham and P.K. Weathersby, Hydrogen bonding. 30. Solubility of gases and vapors in biological liquids and tissues. J. Pharm. Sci. 83, 1450-1456 (1994).

16. M.H. Abraham, M.J. Kamlet, W. Taft, R.M. Doherty and P.K. Weathersby PK, Solubility properties in polymers and biological media: 2. The correlation and prediction of the solubilities of non electrolytes in biological tissues and fluids. J. Med. Chem. 28, 865-870 (1985).

17. A. Morgan, A. Black and D.R. Belcher, Studies on the absorption of halogenated hydrocarbons and their excretions in breath using ^{38}Cl tracer techniques. Ann. Occup. Hyg. 15, 273-282 (1972).

18. A.L. Cowle, H.H. Borgstedt and A.J. Gillies, Solubilities of ethylene, cyclopropane, halothane and diethyl ether in human and dog blood at low concentrations. Anesthesiol. 35, 203-211 (1971).

19. A. Feingold, Estimation of anaesthetic solubility in blood. Anaesthesia Analgesia; Curr. Res. 55, 593-595 (1976).

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

21. A. Sato and T. Nakajima, A structure-activity relationship of some chlorinated hydrocarbons. Arch. Environ. Health 34, 69-75 (1979).

22. Y.C. Pang, P.E. Reid and D.E. Brooks, Solubility and distribution of halothane in human blood. Br. J. Anaesthesia 52, 851-861 (1980).

23. V. Fiserova-Bergerova, M. Tichy and F.J. Di Carlo, Effects of biosolubility on pulmonary uptake and disposition of gases and vapors of lipophilic chemicals. Drug Metab. Rev. 15, 1033-1070 (1984).

24. M. Tichy, V. Fiserova-Bergerova, and F.J. Di Carlo, QSAR in toxicology and xenobiochemistry, ed. M. Tichy, pp. 225-231, Elsevier Sciences Publishers; Amsterdam (1984).

25. M. Tichy, QSAR approach to estimation of the distribution of xenobiotics and the target organ in the body. Drug Metabol. Drug Interact. 9, 191-200 (1991).

26. D.R. Mattie, G.D. Bates, Jr., G.W. Jepson, J.W. Fisher and J.N. McDougal, Determination of skin:air partition coefficients for volatile chemicals: experimental methods and applications. Fund. Appl. Toxicol. 22, 51-57 (1994).

27. L. Perbellini, F. Brugnone, D. Caretta and G. Maranelli, Partition coefficients of some industrial aliphatic hydrocarbons (C5-C7) in blood and human tissues. Br. J. Ind. Med. 42, 172-167 (1985).

28. G.A. Csanady and R.J. Laib, Use of linear free energy relationships in toxicology: prediction of partition coefficients of volatile lipophilics compounds. Arch. Toxicol. 64, 594-596 (1990).

29. K. Yokogawa, E. Nakashima, J. Ishizaki, H. Maeda, T. Nagano and F. Ichimura, Relationships in the structure-tissue distribution of basic drugs in the rabbit. Pharm. Res. 7, 691-696 (1990).

30. R. Cabala, J. Svobodova, L. Felt and M. Tichy, Direct determination of partition coefficients of volatile liquids between oil and gas by gas chromatography and its use in QSAR analysis. Chromatographia 34, 601-606 (1992).

31. F.M. Parham, M.C. Mc Kohn, H.B. Matthews, C. De Rosa and C.J. Portier, Using structural information to create physiologically-based pharmacokinetic models for all polychlorinated biphenyls. Toxicol. Appl. Pharmacol. 144, 340-347 (1997).

32. J. DeJongh, H.J.M. Verhaar and J.L.M. Hermes, A Quantitative property relationship (QSPR) approach to estimate *in vitro* tissue:blood partition coefficients of organic chemicals in rats and humans. Arch. Toxicol. 72, 17-25 (1997).

33. R. Kalizan and M. Markuszewski, Brain/blood distribution described by a combination of partition coefficients and molecular mass. Int. J. Pharm. 145, 9-16 (1996).

34. A. Steward, P.R. Alott, A.L. Cowles and W.W. Mapleson, Solubility coefficients for inhaled anaesthetics for water, oil and biological media. Br. J. Anaesthesiol. 45, 282-293 (1973).

35. J. Lerman, M.M. Willis, G.A. Gregory and E.I. Eger, Osmolarity determines the solubility of anaesthetic in aqueous solutions at 37°C. Anaesthesiol. 59, 554-559 (1973).

36. E.F. Scherertz, K.B. Sloan and R.G. McTiernan , Use of theoretical partition coefficients determined from solubility parameters to predict permeability coefficients for 5-fluorouracil. J. Invest. Dermatol. 89, 147-151 (1987).

37. S.L. Brown and J.E. Rossi, A simple method for estimating dermal absorption of chemicals in water. Chemosphere 19, 1989-2001 (1989).

38. N.E. Tayar, R.S. Tsai, B. Testa, P.A. Carrupt, C. Hansch and A. Leo, Percutaneous penetration of drugs: a quantitative structure-permeability relationship study. J. Pharm. Sci. 80, 744-749 (1991).

39. T.E. McKone and R.A. Howe, Estimating dermal uptake of non ionic organic chemicals from water and soil: I. unified fugacity-based models for risk assessments. Risk Anal. 12, 543-557 (1992).

40. R.H. Guy and R.O. Potts, Penetration of industrial chemicals across the skin: a predictive model. Am. J. Ind. Med. 23, 711-719 (1993).

41. R.O. Potts and R.H. Guy, Predicting skin permeability. Pharm. Res. 9, 663-669 (1992).,

42. R.O. Potts and R.H. Guy, A predictive algorithm for skin permeability : the effects of molecular size and hydrogen bond activity. Pharm. Res. 12, 1628-1633 (1995).

43. W.J. Pugh and J. Hadgraft, Ab initio prediction of human skin permeability coefficients. Int. J. Pharm. 103, 163-178 (1994).

44. W.J. Pugh, M.S. Roberts and J. Hadgraft, Epidermal permeability – penetrant structure relationships : 3. The effect of hydrogen bonding interactions and molecular size on diffusion across the stratum corneum. Int. J. Pharm.. 138, 149-165 (1996).

45. M.S. Roberts, W.J. Pugh, J. Hadgraft and A.C. Watkinson, Epidermal permeability-penetrant structure relationships: 1. An analysis of methods of

predicting penetration of monofunctional solutes from aqueous solutions. Int. J. Pharm. 126, 219-233 (1995).

46. M.S. Roberts, W.J. Pugh and J. Hadgraft, Epidermal permeability: penetrant structure relationships: 2. The effect of H-bonding groups in penetrants on their diffusion through the stratum corneum. Int. J. Pharm. 132, 23-32 (1996).

47. R.L. Bronaught and C.N. Barton, Prediction of human percutaneous absorption with physicochemical data. In Risk assessment. Dermal and inhalation exposure and absorption of toxicants, ed. R.G.M. Wang, J.B. KnaaK and H.I. Maibach, pp. 117-126, CRC Press, Boca Raton (1993).

48. Y. Maitani, A. Coutel-Egros, Y. Obata and T. Nagai, Prediction of skin permeabilities of diclofenac and propanolol from theoretical partition coefficients determined from cohesion parameters. J. Pharm. Sci. 82, 417-420 (1993).

49. M.D. Barratt, Quantitative structure-activity relationships for skin permeability. Toxicol. *In vitro* 9, 27-37 (1995).

50. E.J. Lien and H. Gao, QSAR analysis of skin permeability of various drugs in man as compared to *in vivo* and *in vitro* studies in rodents. Pharm. Res. 12, 583-587 (1995).

51. A. Wilshut, W.F. ten Berge, P.J. Robinson and T.E. McKone, Estimating skin permeation. The validation of five mathematical skin permeation models. Chemosphere 30, 1275-1296 (1995).

52. L.A. Kirchner, R.P. Moody, E. Doyle, J.J. Boser, and I. Chu, The prediction of skin permeability by using physicochemical data. ATLA 25, 359-370 (1997).

53. R.L. Cleek and A.L. Bunge, A new method for estimating dermal absorption from chemical exposure. 1. General approach. Pharm. Res. 10, 497-506 (1993).

54. N. Cnubben, S. Peelen, J.W. Borst, J. Vervoot, C. Veeger, and I. Rietjens, Molecular orbital-based quantitative structure-activity relationship for the cytochrome P450-catalysed 4-hydroxylation of halogenated anilines. Chem. Res. Toxicol. 7, 590-598 (1994).

55. G.M. Cohen and G.J. Mannering, Involvement of a hydrophobic site in the inhibition of the microsomal *p*-hydroxylation of aniline by alcohols. Mol. Pharmacol. 9, 383-397 (1973).

56. Gy A. Csanady, R.J. Laib and J.G. Filser, Metabolic transformation of halogenated and other alkenes – a theoretical approach. Estimation of metabolic reactivities for *in vivo* conditions. Toxicol. Lett. 75, 217-223 (1995).

57. T. Ding and J.M. van Rossum, K_s values of some homologous series of barbiturates and the relationship with the lipophilicity and metabolic clearance. Biochem. Pharmacol. 26, 2117-2130 (1977).

58. C.L. Waller, M.V. Evans and J.D. McKinney, Modeling the cytochrome P450-mediated metabolism of chlorinated volatile organic compounds. Drug Metab. Dispos. 24, 203-210 (1996).

59. J. Ishizaki, K. Yokogawa, E. Nakashima and F. Ichimura, Relationships between the hepatic intrinsic clearance or blood cell-plasma partition coefficients in the rabbit and the lipophilicity of basic drugs. J. Pharm. Pharmacol. 49, 768-772 (1997).

60. A.J.J. Straathof and J.J. Heijen, Derivation of enzymatic rate equations using symbolic software. Biocatalysis Biotransformation 15, 29-37 (1997).

61. L. Szigeti, B. Sevella, J. Rezessy-Szabo and A. Hoschke, Estimation of the turnover number of laccase enxyme. Acta Alimentaria 25, 47-56 (1996).

62. I. Rietjens, E.M.F. Soffers, G. Hooiveld, C. Veeger and J. Vervoort, Quantitative structure-activity relationships based on computer calculated parameters for the overall rate of gluthatione-S-transferase catalysed conjugation of a series of fluoronitrobenzenes. Chem. Res. Toxicol. 8, 481-488 (1995).

63. J.H. Proost, J. Roggeveld, J. Wierda, K.H. Mark and K.F. Meijer Dirk, Relationship between chemical structure and physicochemical properties of series of bulky organic cations and their hepatic uptake and biliary excretion rates. J. Pharmacol. Exp. Ther. 282, 715-726 (1997).

64. K.H. Kim, 3D-Quantitative structure-activity relationships: describing hydrophobic interactions directly from 3D structures using a comparative molecular field analysis (CoMFA) approach. QSAR 12, 232-238 (1993).

65. T. Yamaguchi, M. Yabuki, S. Saito, T. Watanabe, H. Nishimura, N. Isobe, F. Shono and M. Matsuo, Research to develop a predicting system of mammalian subacute toxicity (3) construction of a predictive toxicokinetics model. Chemosphere 33, 2441-2468 (1996).

66. G.E. Blakey, I.A. Nestrov, P.A. Arundel, L.J. Aarons and M. Roeland, Quantitative Structure-Pharmacokinetics Relationships – 1 – Development of a whole body physiologically-based model to characterize changes in pharmacokinetics across a homologous series of barbiturates in the rat. J. Pharmacokinet. Biopharm. 25, 277-312 (1997).

67. H.J. Lowe and K. Hagler, Determination of volatile organic anaesthetic in blood, gases, tissues and lipids: partition coefficients. In: Porter R, ed. Gas

chromatography in biology and medicine. A Ciba-Geigy Foundation Symposium. pp. 86-103, Churchill, New York (1969).

68. A. Falk, E. Gullstrand, A. Löf and E. Wigaeus-Hjelm, Liquid/air partition coefficients of four terpenes. Br. J. Ind. Med. 47, 62-64 (1990).

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

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

71. D.P. Williams and M.A. Tung, Food dispersion. In Principles of food sciences, Part I. Food chemistry, ed. O.R. Fennema, pp. 530-575, Marcel Dekker, New York (1976).

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

73. G. Klopman, C. Ding and O.T, Macina, Computer aided olive oil-gas partition coefficients calculations. J. Chem. Inf. Comput. Sci. 37, 569-575 (1997).

74. P. Poulin and K. Krishnan, A Quantitative Structure-Toxicokinetic Relationship model for highly metabolized chemicals. ATLA 26, 45-49 (1998).

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

76. M. Pelekis, P. Poulin and K. Krishnan, An approach for incorporating tissue composition data into physiologically-based pharmacokinetic models. Toxicol. Ind. Health 11, 511-522 (1995).

77. P. Poulin and K. Krishnan, An algorithm for predicting tissue:blood partition coefficients of organic chemicals from n-octanol:water partition coefficient data. J. Toxicol. Environ. Health 46, 101-113 (1995).

78. R.M. Featherstone, C.A. Muehelbaecher, F.L. DeBon and M.S. Forsaith, Interaction of inert anaesthetic gases with proteins. Anaesthesiol. 22, 977-981 (1961).

79. C.P. Larson, Jr., E.I. Eger, and J.W. Severinghaus, The solubility of halothane in blood and tissue homogenates. Anaesthesiol. 23, 349-355 (1962).

80. J. Janberg and G. Johanson, Liquid/air partition coefficients of the trimethylbenzenes. Toxicol. Ind. Health 11, 81-88 (1995).

81. P. Poulin and K. Krishnan, 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 (1996).

82. G. Johanson and B. Dynésius, Liquid/air partition coefficients of six commonly used glycol ethers. Br. J. Ind. Med. 45, 561-564 (1988).

83. G. Johanson, A. Nihlén and A. Löf A, Toxicokinetics and acute effects of MTBE and ETBE in male volunteers. Toxicol. Lett. 82/83, 713-718 (1995).

84. B. Long, Biochemist's Handbook. E & Spon, London (1961).

85. G.J. Nelson, Lipid composition and metabolism of erythrocytes. In Blood lipids and lipoproteins: quantification, composition and metabolism, ed. G.J. Nelson, pp. 317-386, Wiley, New York (1972).

86. C. Lentner, Geigy scientific tables, Vol. 3, Ciba-Geigy, New Jersey (1981).

87. R.M. Featherstone and B.P. Schoenborn, Protein and lipid binding of volatile anaesthetic agents. Br. J. Anaesthesiol. 36, 150-154 (1964).

88. C.W. Lam, T.J. Gallen, J.F. Boyd and D.L. Pierson, Mechanism of transport and distribution of organic solvents in blood. Toxicol. Appl. Pharmacol. 104, 117-129 (1990).

89. S.H. Brown and D. Hattis, The role of skin absorption as a route of exposure to volatile organic compounds in household tap water: a simulated approach. J. Am. Coll. Toxicol. 8, 839-851 (1989).

90. J.A. Shatkin and H.S. Brown, Pharmacokinetics of the dermal route of exposure to volatile organic chemicals in water; a computer simulation model. Environ. Res. 56, 90-98 (1991).

91. J.W. Nichols, M. McKlm, G.J. Lien and A.D. Hoffmann, Physiologically-based toxicokinetic modeling of three waterborne chloroethanes in rainbow trout. Toxicol.Appl. Pharmacol. 110, 374-389 (1991).

92. B. van Ommen, J. deJongh, J. van de Sandt, B. Blaaudoer, E. Hissink, J. Bogaards and P. van Bladeren, Computer-aided biokinetic modeling combined with *in vitro* data. Toxicol. *In vitro* 9, 537-542 (1995).

93. M.J. de Groot, G.M. Kelder, J.N.M. Commandeur, J.H. van Lenthe and N.P.E. Vermeulen, Metabolites predictions for para-substituted anisoles based on ab initio complete active space self-consistent field calculations. Chem. Res. Toxicol. 8, 437-443 (1995).

94. L. Koymans, N.P.E. Vermeulen, S. van Acker, J.M. Koppele, J.P. Heykants, K. Lavrijsen, W. Meuldermans and G.M. den Kelder, A predictive model for substrates of cytochrome P-450-debrisoquine (2D6). Chem. Res. Toxicol. 5, 211-219 (1992).

95. L. Regan, I.D.L. Bogle and P. Dunnill, Simulation and optimization of metabolic pathways. Comput. Chem. Engineer. 17, 627-637 (1993).

96. G.R. Wilkinson and D.G. Shand, A physiological approach to hepatic drug clearance. Clin. Pharmacol. Ther. 18, 377-390 (1975).

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

98. M.E. Andersen, H.J. Clewell III, and M.L. Gargas. Physiologically-based pharmacokinetic modeling with dichloromethane, its metabolites carbon monoxide and blood carboxyhemoglobin in rats and humans. Toxicol. Appl. Pharmacol. 108, 14-27 (1991).

99. Th. Lavé, S. Dupin, C. Schmitt, B. Valles, G. Ubeaud, R.C. Chou, D. Jaeck and Ph. Coassolo, The use of human hepatocytes to select compounds based on their expected hepatic extraction ratios in humans. Pharm. Res. 14, 152-155 (1997).

100. A.A. Akhrem, D.I. Metelitza, S.M. Bielski, P.A. Kiselev, M.E. Skurko and S.A. Usanov, Mechanism of oxygen activation in hydroxylation reactions involving cytochrome P-450. Croatica Chemica Acta 49, 223-235 (1977).

101. A.A. Akhrem, S.B. Bokut and D.I. Metelitza DI, The nature of the rate-limiting step in aniline hydroxylation involving cytochrome P-450 rat liver microsomes. Chem. Biol. Interact. 18, 195-204 (1977).

102. C. Jung and O. Ristau, Mechanism of the cytochrome P-450 catalysed hydroxylation-thermodynamical aspects and the nature of the active oxygen species. Pharmazie 33, 329-331 (1978).

103. Z. Chan and B.R. Hollobone, A QSAR for stereoidal compound interaction with cytochrome P 4501A1. Environ. Toxicol. Chem. 14, 597-603 (1995).

Table 1. Examples of physiologically-based descriptions used in PBPK models of VOCs

Endpoints	Equations[1]
Arterial blood concentration	$C_a = \frac{Q_p * C_i + Q_c * C_v}{Q_p + (Q_c / P_{b:a})}$
Venous blood concentration	$C_v = \frac{\sum_{t=1}^{n} Q_t * (C_a - C_{vt})}{Q_c}$
Tissue concentration Non-metabolizing tissues	$C_t = \frac{\int_0^t Q_t * (C_a - C_{vt})\ dt}{V_t}$
Metabolizing tissues	$C_t = \frac{\int_0^t Q_t * (C_a - C_{vt}) - \frac{V_{max} * C_{vt}}{K_m + C_{vt}}\ dt}{V_t}$

[1]:C, Q, $P_{b:a}$, V, V_{max}, and K_m refer to concentration, flow rate, blood:air partition coefficient, volume, maximum velocity of metabolism and metabolism affinity constant. Subscripts a, p, i, c, v, vt, and t refer to arterial blood, alveolar ventilation, inhaled air, cardiac blood flow, mixed venous blood, venous blood leaving tissue, and tissue, respectively.

Table 2. Average water and lipid levels in rat tissues [1].

Tissues	Water	Phospholipids	Neutral lipids
Liver	0.70	0.025	0.035
Muscle	0.74	0.01	0.009
Adipose	0.12	0.002	0.853

[1] Data, expressed as fraction of tissue weight, were obtained from Poulin and Krishnan[70].

Table 3. Predicted and experimental rat adipose tissue:air partition coefficients (PCs).

Chemicals	Predicted PCs [1]	Experimental PCs [2]
METHANES		
Difluoromethane	4	1.43 ± 0.31
Fluorochloromethane	19	15.4 ± 1
Bromochloromethane	309	325 ± 3
Dibromomethane	819	792 ± 14
Chlorodibromomethane	2291	1917 ± 165
ETHANES		
Chloroethane	33	38.6 ± 0.7
Hexachloroethane	4281	3321 ± 193
1,2-Dibromoethane	1091	1219 ± 50
1-Bromo-2-chloroethane	487	959 ± 39
1,1,1-Trifluoro-2-chloroethane	21	21.2 ± 0.6
1,1,1-Trifluoro-2-bromo-2-chloroethane	169	182 ± 5
PROPANES		
1-Chloropropane	90	118 ± 2
2-Chloropropane	60	68.4 ± 2
1,2-Dichloropropane	366	499 ± 30
n-Propyl bromide	232	235 ± 6

Iso-Propyl bromide	140	158 ± 5
1-Nitropropane	907	506 ± 33
2-Nitropropane	558	155 ± 4
ETHYLENES		
Vinyl chloride	21	20 ± 0.7
1,1-Dichloroethylene	55	68.6 ± 2.1
Vinyl bromide	48	49.2 ± 1.3
AROMATICS		
Chlorobenzene	1868	1277 ± 43
m-Methyl styrene	12553	11951 ± 692
p-Methyl styrene	11901	11221 ± 972
OTHERS		
Isoflurane	67	98.1 ± 4.6
Allyl chloride	93	101 ± 2
Tricyclodecane	11071	10139 ± 239

[1]: Calculated using data on rat tissue composition,[70] $P_{o:w}$ and $P_{w:a}$ values[7] in Eqn. 3.

[2]: Experimental data determined under *in vitro* conditions were obtained from the literature[7].

Table 4. Predicted and experimental rat liver:air partition coefficients (PCs).

Chemicals	Predicted PCs [1]	Experimental PCs [2]
METHANES		
Difluoromethane	1	2.75 ± 0.39
Fluorochloromethane	3	3.44 ± 0.27
Bromochloromethane	22	29.2 ± 0.5
Dibromomethane	51	68.1 ± 1.4
Chlorodibromomethane	119	126 ±1 7.1
ETHANES		
Chloroethane	2	3.61 ± 0.32
Hexachloroethane	214	369 ± 17.5
1,2-Dibromoethane	67	119 ± 4
1-Bromo-2-chloroethane	31	42.8 ± 3.3
1,1,1-Trifluoro-2-chloroethane	1	1.84 ± 0.14
1,1,1-Trifluoro-2-bromo-2-chloroethane	9	7.62 ± 1.2
PROPANES		
1-Chloropropane	5	5.18 ± 0.38
2-Chloropropane	4	3.15 ± 0.24
1,2-Dichloropropane	20	24.8 ± 2.4
n-Propyl bromide	13	8.17 ± 0.62
Iso-Propyl bromide	8	4.41 ± 0.34

1-Nitropropane	46	153 ± 17
2-Nitropropane	98	62.4 ± 1.4
ETHYLENES		
Vinyl chloride	1	1.6 ± 0.17
1,1-Dichloroethylene	3	4.42 ± 0.30
Vinyl bromide	3	3.33 ± 0.38
AROMATICS		
Chlorobenzene	95	86.3 ± 3
m-Methyl styrene	626	327 ± 23
p-Methyl styrene	594	324 ± 17
OTHERS		
Isoflurane	4	4.07 ± 0.2
Allyl chloride	6	38.9 ± 4.5
Tricyclodecane	551	554 ± 17

[1]: Calculated using data on rat tissue composition,[70] $P_{o:w}$ and $P_{w:a}$ values[7] in Eqn. 3.

[2]: Experimental data determined under *in vitro* conditions were obtained from the literature[7].

Table 5. Predicted and experimental rat muscle:air partition coefficients (PCs).

Chemicals	Predicted PCs [1]	Experimental PCs [2]
METHANES		
Difluoromethane	1	1.44 ± 0.25
Fluorochloromethane	3	2.46 ± 0.52
Bromochloromethane	11	11.1 ± 1.8
Dibromomethane	22	40.5 ± 2
Chlorodibromomethane	38	55.6 ± 0.7
ETHANES		
Chloroethane	1	3.22 ± 0.68
Hexachloroethane	61	75 ± 0.9
1,2-Dibromoethane	28	45.6 ± 3.3
1-Bromo-2-chloroethane	13	25.4 ± 3.1
1,1,1-Trifluoro-2-chloroethane	1	1.23 ± 0.14
1,1,1-Trifluoro-2-bromo-2-chloroethane	3	4.46 ± 0.29
PROPANES		
1-Chloropropane	2	2.08 ± 0.66
2-Chloropropane	1	2.04 ± 0.48
1,2-Dichloropropane	7	12 ± 1.1
n-Propyl bromide	4	4.21 ± 0.32
Iso-Propyl bromide	3	4.12 ± 0.35

1-Nitropropane	14	28.9 ± 6.1
2-Nitropropane	81	29.1 ± 3.3
ETHYLENES		
Vinyl chloride	1	2.1 ± 0.45
1,1-Dichloroethylene	1	2.05 ± 0.35
Vinyl bromide	1	2.26 ± 0.13
AROMATICS		
Chlorobenzene	28	34 ± 3.9
m-Methyl styrene	178	182 ± 10
p-Methyl styrene	169	183 ± 8
OTHERS		
Isoflurane	1	1.6 ± 0.34
Allyl chloride	3	11 ± 0.20
Tricyclodecane	156	674 ± 19

[1]: Calculated using data on rat tissue composition,[70] $P_{o:w}$ and $P_{w:a}$ values[7] in Eqn. 3.

[2]: Experimental data determined under *in vitro* conditions were obtained from the literature[7].

Table 6. Geometric dimensions (in Angstroms), oil:water partition coefficients (Log $P_{o:w}$) and hemoglobin binding characteristics of several organic chemicals.

Chemicals	*Log $P_{o:w}$*	*Length*	*Width*	*Depth*	*Binding*[1]
Methyl alcohol	-1.60	5.05	4.73	4.21	-
Ethyl alcohol	-1.29	6.10	4.63	4.21	-
*Iso*propyl alcohol	-0.99	6.04	5.37	4.75	-
n-Propyl alcohol	-0.79	6.70	5.80	4.12	-
Dimethyl ketone	-0.50	6.10	5.74	4.12	-
n-Butyl alcohol	-0.24	7.98	6.51	4.12	-
Methyl acetate ester	-0.10	7.28	5.64	4.14	-
Methyl ethyl ketone	0.08	7.20	5.74	4.13	-
n-Pentyl alcohol	0.11	9.05	7.46	4.13	-
Ethyl acetate ester	0.39	8.37	6.69	4.13	-
Methyl propyl ketone	0.65	8.49	6.70	4.13	-
Diethyl ether	0.69	7.92	6.69	4.13	-
*Iso*propyl acetate ester	0.95	8.36	6.73	5.39	-
n-Propyl acetate ester	0.98	9.33	6.77	4.13	-
Methyl chloride	0.99	5.24	4.76	4.58	+
Methyl isobutyl ketone	1.11	7.29	6.21	5.39	-
Dichloromethane	1.34	5.90	5.21	4.12	+
1,2-Dichloroethane	1.51	6.79	6.18	4.12	+
Bromochloromethane	1.62	6.15	5.29	4.12	+
Methyl pentyl ketone	1.62	10.31	8.15	4.13	-
n-Butyl acetate ester	1.69	10.63	7.72	4.13	-
1,1-Dichloroethane	1.88	6.04	5.61	5.27	+
Cis-1,2-dichloroethylene	1.93	6.53	5.33	3.46	+
Chloroform	2.08	6.04	5.61	5.27	+
Trans-1,2-dichloroethylene	2.10	6.51	6.44	3.46	+
*Iso*pentyl acetate ester	2.12	10.63	7.71	5.39	-
1,1,2-Trichloroethane	2.13	6.83	6.22	5.27	+
1,2-Dichloropropane	2.19	7.26	5.75	5.23	+
Benzene	2.23	6.97	6.30	3.54	+
1,1,2,2-Tetrachloroethane	2.43	6.43	6.27	6.04	+
1,1,1-Trichloroethane.	2.59	6.43	6.04	5.61	+
Toluene	2.78	7.68	6.97	4.13	+
Trichloroethylene	2.82	6.58	6.52	3.54	+
1,1,1,2-Tetrachloroethane	2.88	6.83	6.43	6.22	+
Chlorobenzene	2.89	7.61	6.97	3.54	+
Carbon tetrachloride	3.03	6.43	6.04	5.90	+
o-Xylene	3.13	7.42	7.39	4.13	+
m-Xylene	3.23	8.31	7.41	4.13	+
p-Xylene	3.27	8.79	7.18	4.13	+
n-Heptane	3.35	11.30	6.11	4.12	+
Styrene	3.40	8.00	7.78	3.54	+
Tetrachloroethylene	3.43	6.63	6.52	3.55	+
Pentachloroethane	3.46	6.87	6.43	6.27	+
n-Hexane	3.78	9.14	7.75	4.13	+
Cyclohexane	4.49	7.00	6.86	5.80	+
Hexachlorobenzene	5.73	9.76	8.93	3.55	-

[1]+ indicates that the hypothesized hemoglobin binding in rats is present .

- signifies the absence of hemoglobin binding for that chemical.

Table 7. Average water and lipid levels in human blood [1].

	Water	Phospholipids	Neutral lipids
Whole blood	0.82	0.0024	0.0033
Plasma	0.94	0.0020	0.0032
Erythrocytes	0.63	0.0020	0.0042

[1] Data, expressed as fraction of blood weight, were obtained from Long[84], Nelson[85] and Lentner[86].

Table 8. Predicted and experimental human blood:air partition coefficients (PCs).

Chemicals	Predicted PCs [1]	Experimental PCs [2]
ALCOHOLS		
Methanol	2737	1625 – 2590
Ethanol	1759	1265 – 1440
n-Propanol	1521	947– 1120
*Iso*Propanol	1233	700 – 848
Butanol	1079	677 ± 79
*Iso*butanol	930	453 – 628
Pentanol	893	534 ± 23
*Iso*Pentanol	702	381 ± 16
1-Methoxypropanol	10093	12383 ± 299
1-Methoxyethanol	29475	32836 ± 1350
2-Ethoxyethanol	18959	22093 ± 538
2-*iso*Propoxyethanol	10153	14416 ± 565
2-Butoxyethanol	5816	7965 ± 61
t- butyl alcohol	496	462
ACETATE ESTERS		
Methyl acetate ester	89	90.1 ± 307
Ethyl acetate ester	59	76.8 ± 3.4
n-Propyl acetate ester	46	73.5 ± 1.7
*Iso*Propyl acetate ester	29	33.1 ± 1.8

Butyl acetate ester	33	83.4 ± 2.9
*Iso*Butyl acetate ester	26	45.1 ± 2.5
n-Pentyl acetate ester	36	92.4 ± 8.4
*Iso*Pentyl acetate ester	30	59.1 ± 3
ETHERS		
Methyl tertiary ether	13	17.7
Methyl tertiary amyl ether	8	11.7
Ethyl *t*-butyl ether	11	17.9
KETONES		
Acetone	180	186 ± 20
2-Butanone	96	125 ± 9
ALCENES		
α-Pinene	12	15 ± 4
β-Pinene	17	23 ± 6
3-Carene	20	32 ± 7
Limonene	24	42 ± 8
AROMATICS		
1,3-Trimethylbenzene	41	40.8 – 45.2
1,2,4-Trimethylbenzene	42	56.9 – 61.3
1,2,3-Trimethylbenzene	46	63.7 – 69.3

[1]:Calculated using average data on human blood composition,[84-86] $P_{o:w}$ and $P_{w:a}$ values[10, 68-69, 80, 82-83] in Eqn. 4.

[2]: Experimental data determined under *in vitro* conditions were obtained from the literature [9-10, 68, 80, 82-83].

Table 9. Predicted and experimental human erythrocyte:air and plasma:air partition coefficients (PCs).

Chemicals	Erythrocyte:air PCs		Plasma:air PCs	
	Predicted[1]	Experimental[2]	Predicted[1]	Experimental[2]
Methanol	2106	1522 ± 265	3135	1871 ± 172
Ethanol	1353	1246 ± 215	2015	1455 ± 115
n-Propanol	1171	799 ± 99	1743	969 ± 60
*Iso*Propanol	950	605 ± 68	1413	812 ± 56
*Iso*Butanol	716	519 ± 106	1066	591 ± 154
Acetone	139	170 ± 29	206	217 ± 14
2-Butanone	74	106 ± 10	110	133 ± 11

[1]: Calculated using average data on erythrocyte and plasma composition[84-86], $P_{o:w}$ and $P_{w:a}$ values[10, 69] in Eqn. 4.

[2]: Experimental data determined under *in vitro* conditions were obtained from the literature[9].

FIGURE LEGENDS

Figure 1 Ratio of predicted/experimental rat liver:air (l), muscle:air (m), and fat:air (f) partition coefficients for alcohols (l: 0.84 ± 0.22, m: 0.99 ± 0.2, f: 0.91 ± 0.07, n=6), ketones (l: 0.72 ± 0.25, m: 0.96 ± 0.11, f: 1.27 ± 0.17, n=5), acetate esters (l: 0.45 ± 0.25, m: 0.59 ± 0.39, f: 1.29 ± 0.66, n=6), alkanes (l: 1.33 ± 0.2, m: 1.6 ± 1.2, f: 1.02 ± 0.12, n=3) haloalkanes (l: 0.99 ± 0.3, m: 0.9 ± 0.25, f: 1.03 ± 0.27, n=17), and some aromatic hydrocarbons (l: 1.22 ± 0.35, m: 0.81 ± 1.18, f: 1.25 ± 0.38, n=7). Data from Poulin and Krishnan[69].

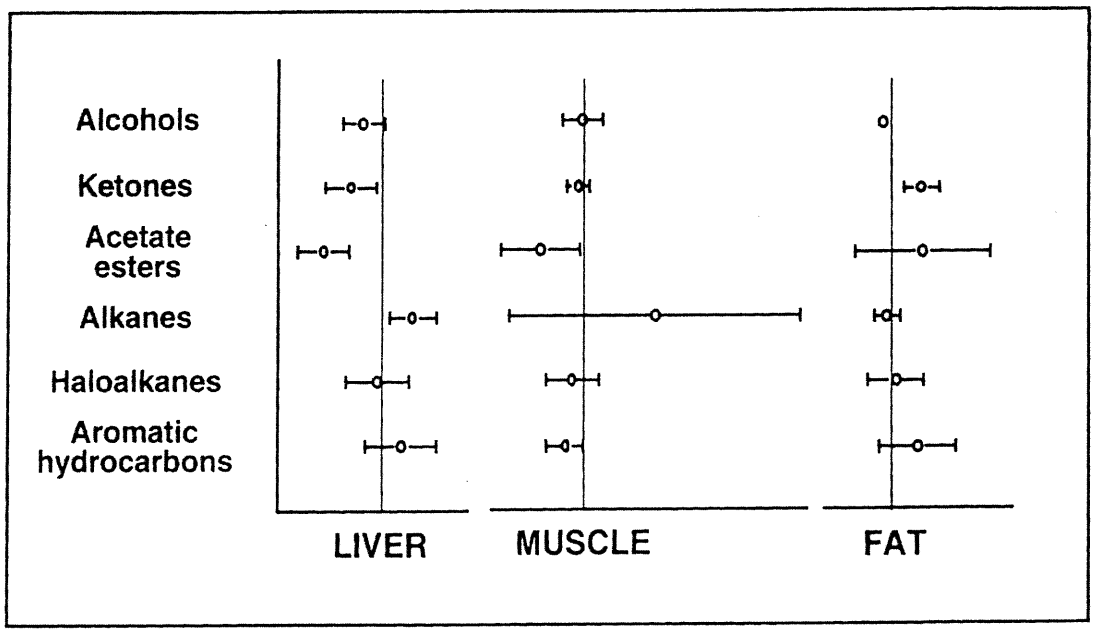
Figure 2 Ratio of predicted/experimental rat blood:air partition coefficients for alcohols (0.96 ± 0.14), ketones (0.89 ± 0.1), acetate esters (0.63 ± 0.23), alkanes (0.25 ± 0.16), haloalkanes (0.21 ± 0.06), and some aromatic hydrocarbons (0.18 ± 0.03). Data from Poulin and Krishnan[69].

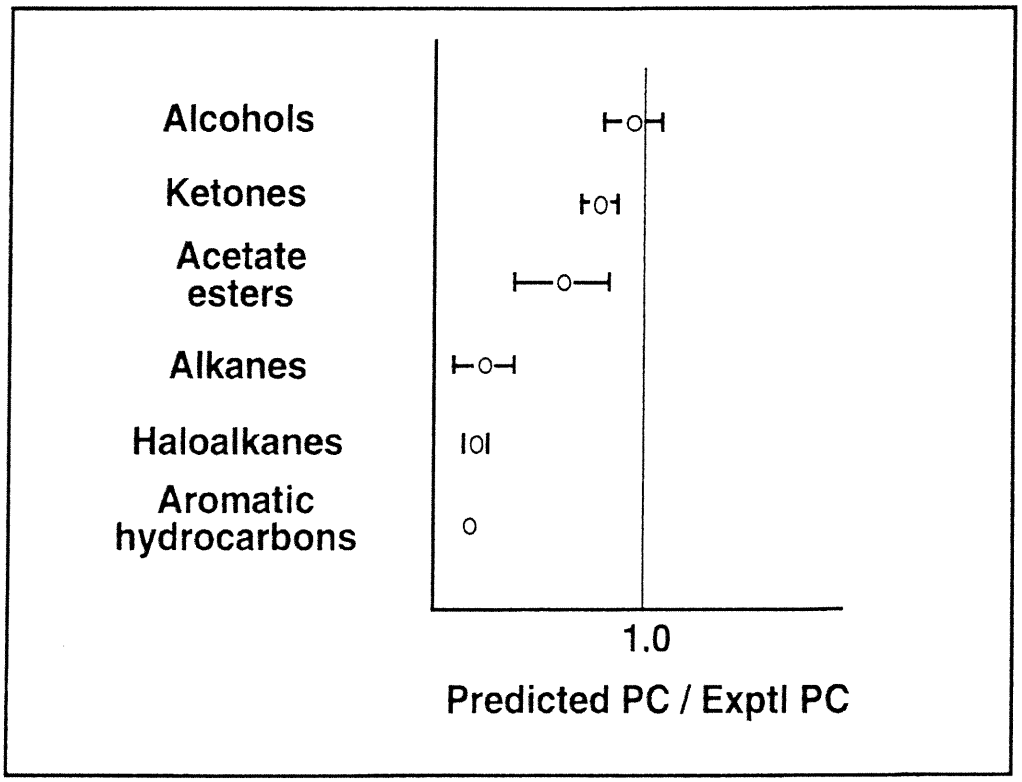
Figure 3 Geometric volumes and log oil:water partition coefficients of chemicals that have been hypothesized to bind to rat hemoglobin (open circles) and that do not bind to rat hemoglobin (closed circles). Data from Table 6.

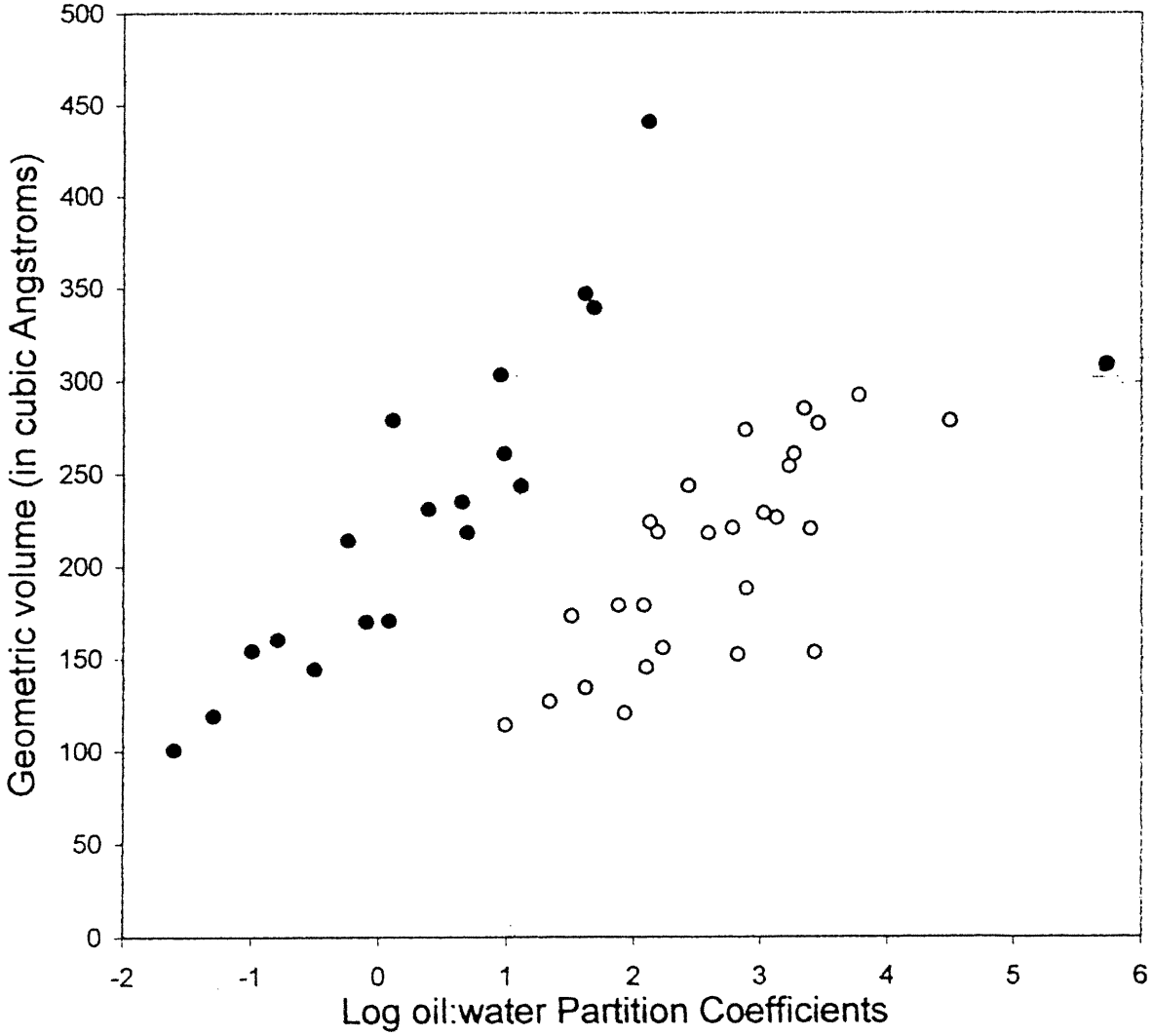
Figure 4 Comparison of the PBPK model simulations (solid lines) of arterial blood concentration of styrene with experimental data (symbols)

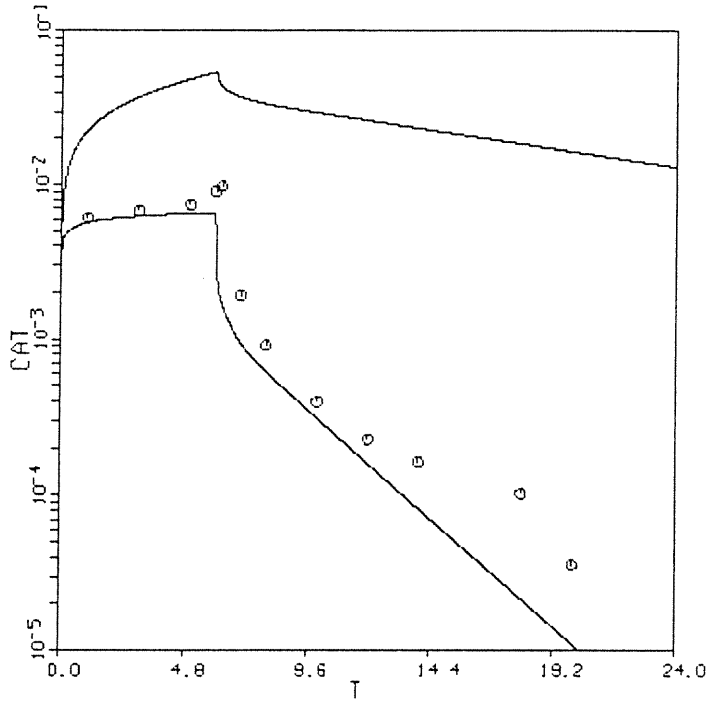
obtained in rats exposed to 80 ppm for 6 hr. Simulations were obtained using tissue:air PCs and blood:air PCs predicted using Eqns. 3 and 9, and by setting E value to 0 (upper line) or 1 (lower line). Physiological parameters were obtained from Ramsey and Andersen[97].

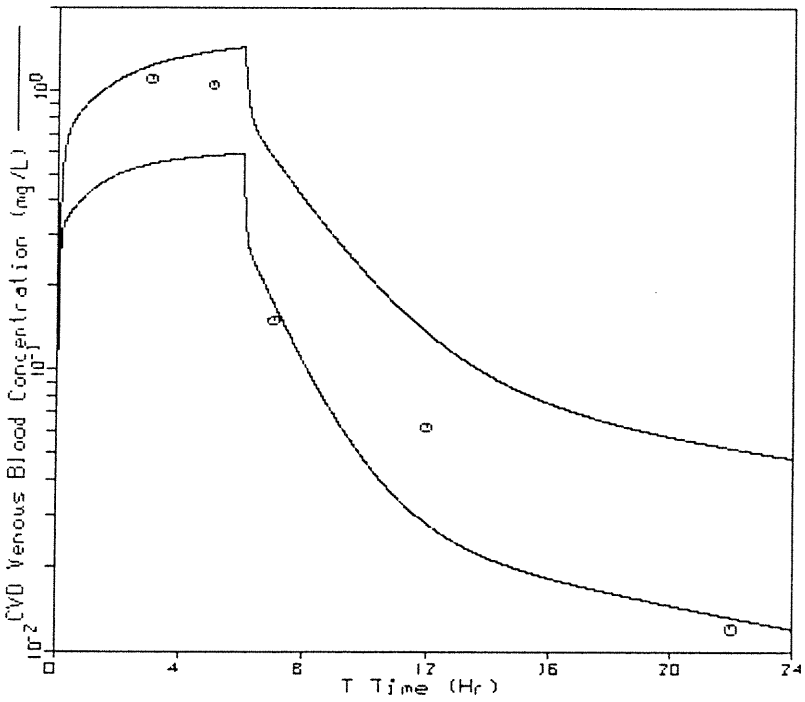
Figure 5 Comparison of the PBPK model simulations (solid lines) of venous blood concentration of dichloromethane with experimental data (symbols) obtained in humans exposed to 100 ppm for 6 hr. Simulations were obtained using tissue:air PCs and blood:air PCs predicted using Eqns. 3 and 4, and by setting E value to 0 (upper line) or 1 (lower line). Physiological parameters were obtained from Andersen et al.[98].


Alcohols
Ketones
Acetate esters
Alkanes
Haloalkanes
Aromatic hydrocarbons
LIVER
MUSCLE
FAT


Alcohols
Ketones
Acetate esters
Alkanes
Haloalkanes
Aromatic hydrocarbons
1.0
Predicted PC / Exptl PC


Geometric volume (in cubic Angstroms)
500
450
400
350
300
250
200
150
100
50
0
-2
-1
0
1
2
3
4
5
6
Log oil:water Partition Coefficients


10⁻¹
10⁻²
10⁻³
10⁻⁴
10⁻⁵
[A]
0.0
4.8
9.6
14.4
19.2
24.0
T


CVO Venous Blood Concentration (mg/L)
10^0
10^-1
10^-2
0
4
8
12
16
20
24
T Time (Hr)

CHAPITRE TROISIÈME :

3 – ARTICLE II

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DETERMINANTS OF BLOOD PROTEIN BINDING OF VOLATILE ORGANIC CHEMICALS

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KEYWORDS: Hemoglobin, Binding, Blood:air partition coefficients, VOCs, Pharmacokinetics, PBPK modeling.

ABSTRACT

The rat blood:air partition coefficients (PC) of some volatile organic chemicals (VOCs) predicted on the basis of lipid and water solubility alone are lower than the experimental blood:air PC ($P_{b:a}$) values. This discrepancy has been attributed to blood protein binding. Analysis of the various critical determinants of protein binding suggested that only chemicals without an oxygen atom in their structure, having a log oil:water PC > 1, and a geometric volume < 300 $Å^3$ would display binding. The objectives of the present study were therefore to: (i) experimentally validate the predictive use of these critical determinants of blood protein binding of VOCs, and (ii) experimentally evaluate the contribution of hemoglobin (Hb) to the $P_{b:a}$ of selected VOCs [butyl methyl ether (BME), *t*-butyl methyl ether (tBME), diethyl ether (ETH), *iso*octane (ISO), methyl ethyl ketone (MEK), α-pinene (PIN), bromoform (BF), chlorobenzene (CB) chloroform (CF), and ethylbenzene (EB)]. Vial equilibration studies showed that the matrix:air PCs were similar for rat blood and oil+water samples (mean±SE, n=7-8) for BME (6.64±1.4 vs 11.1±2.0), tBME (15.0±4 vs 15.0±2), ETH (9.24±0.75 vs 9.53 ±1.16), ISO (1.92±0.4 vs 2.88±0.5), MEK (139±6 vs 159.3±8), and PIN (16.9±1.8 vs 20.5±2.7), whereas the PCs displayed a marked difference for BF (161±5 vs 28.9±3.4), CF (16.9±1.1 vs 3.4±0.75), CB (61.8±2.8 vs 8.3±2.35), and EB (50.8±1.3 vs 7.13±1.6). These results confirm that the additional consideration of binding to blood proteins is essential to adequately determine rat $P_{b:a}$ of BF, CB, CF, and EB. Experimental evaluations of the contribution of Hb binding to the $P_{b:a}$ of these VOCs were therefore performed. The PCs of VOCs determined using whole blood were

comparable to those obtained using a reconstituted mixture of *n*-octanol, water and Hb (mean±SE, n=3-4) for BF (154±1.5), CB (55±6), CF (15±0.87), and EB (30±1.5). The blood protein binding of VOCs as experimentally verified in this study is fundamental to the conduct of scientifically sound extrapolations of the high dose to low dose behavior of VOCs using PBPK models.

INTRODUCTION

The blood:air partition coefficient (PC) is an important determinant of the absorption, distribution, and elimination of volatile organic chemicals (VOCs). The pharmacokinetics (PK) of VOCs can differ depending on whether chemicals possess a relatively high (e.g., methanol) or low (e.g., styrene) blood:air PC. Physiologically-based pharmacokinetic (PBPK) models incorporate blood:air PCs alongwith other physicochemical, biochemical, and physiological parameters in order to predict the PK of VOCs (Krishnan and Andersen, 1994).

The blood:air PCs of VOCs are frequently determined *in vitro* using a vial equilibration technique (Sato and Nakajima, 1979; Fiserova-Bergerova, 1983; Perbellini *et al.*, 1985; Fiserova-Bergerova and Diaz, 1986; Johanson and Dynesius, 1988; Gargas *et al.*, 1989; Kaneko *et al.*, 1994; Tardif *et al.*, 1997). Recently, an algorithm that considers the solubility within the water and lipid fractions of blood was developed in order to estimate rat and human blood:air PCs of VOCs (Poulin and Krishnan, 1996b). Although this approach accurately predicted the rat blood:air PCs of relatively hydrophilic VOCs (i.e. alcohols, ketones, and esters), a large discrepancy between predicted and experimental rat blood:air PCs appeared when the same approach was applied to relatively lipophilic VOCs (i.e. alkanes, haloalkanes, and aromatics). Although never verified experimentally, this discrepancy was speculated to be due to binding of these lipophilic VOCs to hydrophobic pockets within rat hemoglobin, and association

constants (K_a) were subsequently derived based on theoretical considerations (Poulin and Krishnan, 1996a).

Even though the binding of drugs or anesthetics to human blood proteins has been extensively studied (Brown *et al.*, 1976; Wilkinson, 1983; Lindup, 1987; Fichtl *et al.*, 1991; Eckenhoff and Johansson, 1997; Hinderling, 1997), the binding of VOCs to rat blood proteins and its impact on PK, have not been investigated adequately. Since both human serum albumin and hemoglobin have been shown to bind to several anesthetics (Johansson, 1997), they are both likely candidates for the binding of VOCs in rat blood. Furthermore, it is known that dichloromethane binds to hemoglobin S and therefore prevents sickle cell anemia (Schoenborn, 1976). In fact, there is evidence that lipophilic VOCs partition preferably into the erythrocyte fraction of rat blood, whereas hydrophilic VOCs partition equally between the plasma and erythrocytes (Lam *et al.*, 1990). Although this observation suggests that hemoglobin may be the major protein implicated in the binding of VOCs in rat blood, an experimental evaluation to this effect is lacking.

Recently, Poulin *et al.* (1999) identified the critical determinants for the blood protein binding of VOCs that would enable *a priori* prediction of VOCs that could potentially bind to the hydrophobic pockets in hemoglobin. These authors proposed that only VOCs that do not contain an oxygen in their molecule will exhibit binding provided their log oil:water partition coefficient (log P) is at least 1 and their geometric volume (GV) does not exceed 300 $Å^3$. The identification of these critical determinants of blood protein binding of VOCs resulted from a systematic,

theoretical analysis of the available experimental data and solubility-based predictions of rat blood:air PCs of several VOCs (Poulin *et al.*, 1999). Experimental validation of the predictions of blood protein binding of VOCs based on these critical determinants is yet to be performed.

The objectives of the present study were therefore to: (i) experimentally validate the predictive use of critical determinants of the blood protein binding of VOCs, and (ii) experimentally evaluate the contribution of hemoglobin binding to the blood:air PCs of selected VOCs.

MATERIALS AND METHODS

A total of 10 VOCs were chosen for the present study (Table 1). According to the critical determinants identified by Poulin *et al.* (1999), the role of blood protein binding should be negligible for six of these VOCs (butyl methyl ether, *t*-butyl methyl ether, diethyl ether, *iso*octane, methyl ethyl ketone, and α-pinene), which either have an oxygen atom in their molecule, a log P less than 1, and/or a geometric volume exceeding 300 $Å^3$ (Table 1). Hypothetically, for these VOCs then the consideration of solubility in blood lipids and water should be sufficient for estimating their blood:air PC. Based on the molecular characteristics (log P > 1, absence of oxygen atom in the molecule and geometric volume less than 300 $Å^3$), protein binding was anticipated to be important in the determination of the blood:air PCs of the four other VOCs (bromoform, chlorobenzene, chloroform, and ethylbenzene).

The overall approach consisted of comparing the experimental rat blood:air PCs of VOCs to their PCs that were determined between air and a matrix composed of a volume of water and lipids equivalent to what is present in rat blood. Such comparisons were made for several VOCs in order to validate, refute, or refine the critical determinants that have been suggested by Poulin *et al.* (1999) to facilitate the identification of VOCs for which protein binding is likely to be of importance in predicting rat blood:air PC. When the experimentally determined whole blood:air PCs and lipid + water matrix:air PCs are comparable, it would suggest that the role of protein binding is negligible and that rat blood:air PCs can be predicted based

on the sole consideration of solubility in the lipid and water components of rat blood (Poulin *et al.*, 1996b).

I. Experimental determination of PCs using whole blood or mixtures of lipids and water

Chemicals

Bromoform (99%), butyl methyl ether (99%), *tert*-butyl methyl ether (99.8%), chlorobenzene (99%), chloroform (99.9%), diethyl ether (98+%), ethylbenzene (99%), *iso*octane (99+%), methyl ethyl ketone (99+%), and α-pinene (98%) were purchased from Aldrich Chemical Co. (Oakville, Ontario, Canada).

Animals

Male Fischer 344 rats (190-250g) were purchased from Charles River Canada, Inc. (St-Constant, Quebec, Canada). The rats were housed in groups of three in stainless steel cages and maintained on a 12-hour light/dark cycle under controlled temperature and relative humidity. Food and water were provided *ad libitum* throughout an acclimation period of 7 days. Rats were then sacrificed following anesthesia with CO_2, and heparinized blood from the animals was collected and pooled.

Matrices

The matrices used in the experimental determination of PCs include pooled whole blood (0.25-0.50 ml) obtained as described above, as well as commercially available corn oil and water. The volumes of oil (0.50-1.0 μl) and water (211-422 μl) used in these experiments correspond to the volumes of neutral lipid-like components and water-like components reported to be present in 0.25-0.50 ml rat blood (Poulin and Krishnan, 1996b).

Experimental set-up

PCs were determined by vial equilibration technique as described by Gargas *et al.* (1989), and modified by Tardif *et al.* (1997). The experimental procedure for determining the PCs involved: (i) introducing an average of 0.138 μmol of each chemical into sealed glass vials (22 ml) that were either empty (i.e., reference vials) or containing the matrices of interest (0.25 ml of whole blood, or a mixture of corn oil and water), and (ii) quantifying the headspace concentrations of chemicals at 1 hr and 2 hr following their introduction into the vials. All vials were placed in a shaker-incubator and maintained at 37°C throughout the experimental procedure.

Chemical analysis

The concentrations of VOCs in the headspace of the glass vials were determined by injecting 1 ml of the headspace with a TEKMAR® headspace Autosampler 7000 into a gas chromatograph (Hewlett Packard 5890) equipped with a flame ionization

detector. Separation and quantification of products were performed using a HP-608 column, 30 m I.D., 0.5 mm, 0.5 μm film thickness (megabore), with helium (5 ml/min) as the carrier gas. Detector temperature was 250°C and column temperature was 55°C. Oven temperature settings were adjusted between 55°C (for the more volatile, e.g., diethyl ether) and 140°C (for the less volatile, e.g., bromoform) in order to keep retention times close to 3 minutes.

Data analysis

Using the headspace concentrations of the chemicals determined at equilibrium, oil+water:air and blood:air PCs were calculated as follows (Gargas *et al.*, 1989):

$$\text{matrix: air PC} = \frac{C_r \cdot V_r - C_s \cdot (V_r - V_s)}{C_s \cdot V_s} \qquad [1]$$

where C_r = Chemical concentration in the empty, reference vial

C_s = Chemical concentration in the headspace of the vial containing the matrix

V_r = Volume of the reference vial, and

V_s = Volume of the sample

Comparison of blood:air and oil+water:air PCs

Results of the experimentally determined blood:air PC and oil+water:air PC were compared by Student-*t* test in order to determine statistical significance ($p \leq 0.05$). Protein binding was assumed to be present when a statistically significant difference between experimentally determined oil+water:air PCs and blood:air PC was observed.

II. Contribution of hemoglobin binding to the blood:air PC

When protein binding was present for the VOCs, the role of hemoglobin was evaluated for these chemicals. The experimental procedure was identical to the one presented above, except that the oil+water mixture matrix was replaced with an *n*-octanol (99.99%, Fischer Scientific Ltd, Nepean, Ontario, Canada) and water mixture, to which was added rat hemoglobin (Hb) (Sigma-Aldrich, Oakville, Ontario, Canada) in order to yield a physiological concentration (2.34 mM) (Poulin and Krishnan, 1996a). The headspace concentrations were analysed to determine the PCs, according to Eqn. [1]. The hemoglobin+water+*n*-octanol:air PC of each VOC was then compared to its whole blood:air PC.

RESULTS

Experimentally determined oil+water:air PCs and whole blood:air PCs of ten chemicals, as well as their ratios are presented in Table 2. Both PCs were similar for butyl methyl ether, *t*-butyl methyl ether, diethyl ether, *iso*octane, methyl ethyl ketone, and α-pinene, and the ratio of their oil+water:air PC/blood:air PC is ≥ 1 for all 6 chemicals (Table 2). Therefore for these VOCs, the blood:air PC is adequately determined by the solubility in blood lipids and water. However, when the experimental blood:air PCs and oil+water:air PCs for BF, CB, CF, and EB are compared, a statistically significant difference between the PCs is observed as the oil+water:air PC/blood:air PC ratio is < 1. In fact, for these chemicals, solubility in lipids and water accounts for only about 10 to 20% of the whole blood:air PC and binding to blood protein must additionally be considered to adequately determine their blood:air PC values. Based on the hypothesis that Hb was the major ligand of VOCs in rat blood (Lam *et al.*, 1990), the contribution of Hb to the blood:air PCs of these four VOCs was evaluated. The PCs of VOCs determined using whole blood were comparable to those obtained using a reconstituted mixture of *n*-octanol, water and Hb (mean±SE, n=3-4) for bromoform(154±1.5), chlorobenzene (55±6), chloroform (15±0.87), and ethylbenzene (30±1.5) (Table 3). The ratio of reconstituted blood mixture:air PC to whole blood:air PC approached unity for three of the four chemicals investigated (Table 3), indicating that Hb binding contributes significantly to their blood:air PCs.

DISCUSSION

Recently, algorithms have been developed for predicting blood:air PCs of VOCs from data on their solubility in the lipid and water fractions of blood (Poulin and Krishnan, 1996a,b). However, as in the case of rats, when binding to blood proteins occurs, consideration of solubility in water and lipid alone is inadequate for estimating the blood:air PC (Poulin and Krishnan, 1996a). Although never demonstrated experimentally, binding to rat blood proteins has been suspected to be responsible for the discrepency between experimental values and solubility-based predictions of blood:air PC (Poulin and Krishnan, 1996a). Because of the possible impact on the dose-dependency of VOCs, it is essential to verify whether chemicals display binding and whether that phenomenon contributes significantly to the numerical value of blood:air PC.

A previous study (Poulin *et al.*, 1999) identified three mechanistic determinants of the possible VOC binding to blood proteins. Even though these determinants suggest that relatively small lipophilic molecules partition preferably into the erythrocytes and are consistent with data found in the literature (Lam *et al.*, 1990), experimental evidence is lacking for the validation of the predictive ability of these determinants. The present study addressed this lacuna by comparing the blood:air PCs of VOCs determined using whole blood and a matrix composed of water and oil found in whole blood. In case of chemicals with a GV < 300 $Å^3$, a log P < 1, and an oxygen in their molecule, the ratios of PCs estimated using whole blood and reconstituted oil/water mixture approached unity. However, in the case of

chemicals which lack an oxygen and have a smaller GV, the ratio of the reconstituted mixture to whole blood PCs was smaller than 1, indicating that solubility in lipid and water cannot account for the entire value of the blood:air PC. However, when this lipid+water was supplemented with rat Hb, a ligand hypothesized to be responsible for VOC binding in rat blood, this ratio approached unity, indicating that solubility or binding in hemoglobin accounts for the observed discrepancy between whole blood PCs and lipid+water PCs.

The binding could be related to the lipophilicity of the chemical, as it has been shown that all molecules that display binding have a log P > 1. There is evidence in the literature of the presence of hydrophobic domains or pockets within both human hemoglobin and albumin (Eckenhoff and Johansson, 1997) that bind to drugs and anesthetics. Due to the general interspecies similarities between blood proteins (Lindup, 1987), it is reasonable to assume that these pockets can exist within rat hemoglobin. The presence of oxygen can be a determining factor since, contrary to the aromatics, haloalkanes, and alkanes, chemicals containing oxygen (ketones, alcohols and esters) have the potential of forming hydrogen bonds with water molecules (Eckenhoff and Johansson, 1997). An oxygen surrounded by water molecules could have an impact on the global GV of the molecule. The water-VOC GV would be much greater than the VOC GV, possibly greater than the limit of 300 $Å^3$. This chemical-water complex could exert steric hindrance, and would therefore not fit in the hydrophobic pocket, despite possessing the lipophilic character, or the water hydration simply lowers the lipophilicity of the compound below the log P threshold of 1. The effective threshold of lipophilicity would have to

be higher in order to break free of the hydrogen bond and solubilize in the pocket. Further studies that examine the effect of replacing the oxygen with another less electrophilic atom (i.e. nitrogen), could shed light on the influence of hydrogen bonds on the binding to blood proteins.

The fact that molecules with a GV > 300 $Å^3$ do not display binding is not surprising since a physical molecular size limit for blood protein binding is possible (Schoenborn, 1968; Schoenborn, 1976; Eckenhoff and Johansson, 1997). The fact that for 6 of the 10 VOCs investigated in the present study, there is no marked discrepancy between the whole blood and oil+water PCs suggests that the solubility in the water and lipid fractions of rat blood are the only mechanistic determinants involved in the blood:air PC and protein binding is minimal for these chemicals. If the log P > 1, GV < 300 $Å^3$, and the chemical contains no oxygen (4 of the 10 chemicals investigated in the present study), then a discrepancy occurs and blood protein binding must be considered in addition to lipid and water solubility as a mechanistic determinant of the blood:air PC.

Although studies by Lam *et al.* (1990) suggest hemoglobin is the major transporter molecule of VOCs in rat blood, the relative contribution of Hb to the blood:air PC has never been investigated previously. The results of the present study confirm that a reconstituted mixture of *n*-octanol, water, and Hb can adequately represent the blood:air PC. The mixture of water, lipid and proteins was able to account for 86%, 96%, 88%, and 60% of the value of the blood:air PC for bromoform, chlorobenzene, chloroform, and ethylbenzene respectively. Hb is therefore likely to

be the protein in rat blood that contributes the most to the blood:air PCs of VOCs. However, other blood proteins such as albumin, have the potential of demonstrating an important role (up to 40% in the case of ethylbenzene) and this aspect should be verified experimentally. Overall, the present study demonstrated that a mixture of water and oil adequately describes the blood:air PC of hydrophilic VOCs whereas the additional consideration of the role of proteins was essential to approximate the blood:air PC of lipophilic VOCs.

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REFERENCES

Brown, F.F., Halsey, M.J., Richards, R.E., 1976. Halothane interactions with haemoglobin. Proc. R. Soc. Lond. B: Biol. Sci. 193, 387-411.

Eckenhoff, R.G., Johansson, J.S., 1997. Molecular interactions between inhaled anesthetics and proteins. Pharmacol. Rev. 49, 343-367.

Fichtl, B., Nieciecki, A.V., Walter, K., 1991. Tissue binding versus plasma binding of drugs: general principles and pharmacokinetic consequences. Adv. Drug Res. 20, 117-166.

Fiserova-Bergerova, V., 1983. Gases and their solubility: a review of fondamentals. In: Fiserova-Bergerova, V. (Ed.), Modeling of Inhalation exposure to vapors: uptake, distribution and elimination. CRC Press, Boca Raton, pp. 3-28.

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

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.

Hinderling, P.H., 1997. Red blood cells: a neglected compartment in pharmacokinetics and pharmacodynamics. Pharmacol. Rev. 49, 279-295.

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

Johansson, J.S., 1997. Binding of the volatile anesthetic chloroform to albumin demonstrated using tryptophan fluorescence quenching. J. Biol. Chem. 272, 17961-17965.

Kaneko, T., Wang, P.Y., Sato, A., 1994. Partition coefficients of some acetate esters and alcohols in water, blood, olive oil, and rat tissues. Occup. Environ. Med. 51, 68-72.

Krishnan, K., Andersen, M.E., 1994. Physiologically based pharmacokinetic modeling in toxicology. In: Hayes, W. A. (Ed.), Principles and Methods of Toxicology, 3rd Ed., Raven Press, New York, pp. 149-188.

Lam, C.W., Galen, T.J., Boyd, J.F., Pierson, D.L., 1990. Mechanism of transport and distribution of organic solvents in blood. Toxicol. Appl. Pharmacol. 104, 117-129.

Lindup, W.E., 1987. Plasma protein binding of drugs - some basic and clinical aspects. Prog. Drug Metab. 10, 141-185.

Long, C., 1961. Biochemist's Handbook, E & F Spon Ltd, London.

Perbellini, L., Brugnone, F., Caretta, D., Maranelli, G., 1985. Partition coefficients of some industrial aliphatic hydrocarbons (C5-C7) in blood and human tissues. Br. J. Ind. Med. 42, 162-167.

Poulin, P., Krishnan, K., 1996a. 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.

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

Poulin, P., Béliveau, M., Krishnan, K., 1999. Mechanistic animal-replacement approaches for predicting pharmacokinetics of organic chemicals. In: Salem, H., Katz, S.A. (Eds.), Toxicity Assessment Alternatives: Methods, Issues, Opportunities, Humana Press, Totowa, pp 115-139.

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

Schoenborn, B.P., 1968. Binding of anesthetics to protein: an x-ray crystallographic investigation. Fed. Proc. 27, 888-894.

Schoenborn, B.P., 1976. Dichloromethane as an antisickling agent in sickle cell hemoglobin. Proc. Natl. Acad. Sci. USA 73, 4195-4199.

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.

Wilkinson, G.R., 1983. Plasma and tissue binding considerations in drug disposition. Drug Metab. Rev. 14, 427-465.

Table 1: Chemicals used in this study, their geometric volumes (GV), log oil:water partition coefficients (log P), and the presence of their binding to blood proteins

Chemicals	*log P*	*GV ($Å^3$)*	*Oxygen*[a]	*Binding to blood proteins*[b]
Butyl methyl ether	>1	<300	+	-
t-Butyl methyl ether	>1	<300	+	-
Diethyl ether	<1	<300	+	-
Methyl ethyl ketone	<1	<300	+	-
Isooctane	>1	>300	-	-
α-Pinene	>1	>300	-	-
Bromoform	>1	<300	-	+
Chlorobenzene	>1	<300	-	+
Chloroform	>1	<300	-	+
Ethylbenzene	>1	<300	-	+

[a] + denotes the presence of oxygen within the molecule

[b] + denotes the presence of blood protein binding

Table 2: Blood:air partition coefficients (PC) and oil+water:air partition coefficients of the VOCs used in this study

Chemical	*Blood:air PC*[a]	*Oil+water:air PC*[a,b]	*Ratio of experimental PCs*
Butyl methyl ether	6.64±1.4	11.1±2.0	1.67
t-Butyl methyl ether	15.0±4	15.0±2	1.00
Diethyl ether	9.24±0.75	9.50±1.16	1.03
Isooctane	1.92±0.4	2.88±0.48	1.5
Methyl ethyl ketone	139±6	159±8	1.14
α-Pinene	16.9±1.8	20.5±2.7	1.21
Bromoform	161±5	19.0±3.4*	0.12
Chlorobenzene	61.8±2.8	8.30±2.4*	0.13
Chloroform	16.9±1.10	3.40±0.75*	0.20
Ethylbenzene	50.8±1.26	7.13±1.57*	0.14

[a] : Values represent mean ± standard error (n = 3-8)

[b] : *indicates statistical significance.

Table 3: Partition coefficient (PC) between a reconstituted blood mixture composed of *n*-octanol, water and hemoglobin (Hb) and air for chemicals that displayed binding in this study

Chemical	*Mixture:air PC of the n-octanol, water and Hb mixture*[a]	*Ratio of the mixture:air PC/experimental whole blood:air PC*[b]
Chlorobenzene	55±6	0.89
Chloroform	15±0.87	0.89
Bromoform	154±1.5	0.96
Ethylbenzene	30±1.5	0.59

[a] : values are mean ± S.E. (n = 3-4)

[b] : values for blood:air PC taken from Table 2.

CHAPITRE QUATRIÈME :

4 – ARTICLE III

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Experimental estimation of the rat blood protein association constants of several volatile organic chemicals

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Running title: Determining the blood protein K_a for VOCs

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ABSTRACT

The rat blood:air partition coefficient (PC) of lipophilic volatile organic chemicals (VOCs) cannot be predicted with the sole consideration of their solubility in blood water and lipids, suggesting an important role of protein binding. Previous studies have determined the importance of protein binding for bromoform (BF), chlorobenzene (CB), chloroform (CF), and ethylbenzene (EB). The possible dose-dependency and the quantitative nature of VOC-binding to hemoglobin [i.e., association constant (K_a), number of binding sites (n)] have not been investigated previously. The objectives of this study were therefore: i) to determine the dose-dependency of the blood:air PC ($P_{b:a}$) of four VOCs (BF, CF, CB, and EB) known to display binding to rat blood proteins, and ii) to derive K_a and n values for the binding of these chemicals to rat blood proteins. *In vitro* vial equilibration studies were conducted using 0.1-0.5 ml whole blood, or an equivalent mixture of water and *n*-octanol exposed to varying amounts of VOCs [bromoform (BF, 0.11 to 11.4 μmol), chlorobenzene (CB, 0.11 to 24.6 μmol), chloroform (CF, 0.11 to 186.6 μmol), and ethylbenzene (EB, 0.11 to 20.2 μmol)]. The $P_{b:a}$ of all four VOCs decreased significantly at higher amounts added, with no significant change in their *n*-octanol+water mixture:air PC. For each *in vitro* exposure situation, the concentration of free chemical (C_{free}) in rat blood was calculated with the PC for the *n*-octanol+water mixture, whereas the concentration of bound plus free chemical (C_{tot}) was calculated from knowledge of the PC determined experimentally with whole blood. The values of K_a (mM^{-1}) and n for hemoglobin binding estimated by linear regression of a plot of the reciprocal of the molar ratio of bound chemical vs

$1/C_{free}$ were: BF (0.8, 4), CB (2.8, 1.4), CF (1.8, 1.2), and EB (2, 1.4). The results of this study suggest that the dose-dependent nature of $P_{b:a}$ need not be considered for modeling inhalation exposures to these VOCs for up to 2000-5000 ppm.

Keywords: Blood protein binding, VOCs, PBPK models, blood:air PC, hemoglobin binding.

INTRODUCTION

The blood:air partition coefficient ($P_{b:a}$), a physico-chemical parameter widely used in physiologically-based pharmacokinetic (PBPK) modeling (Krishnan and Andersen, 1994), is an important determinant of the pharmacokinetics of volatile organic chemicals (VOCs). Animal-replacement algorithms have recently been developed for predicting the rat and human $P_{b:a}$ of VOCs from information on the solubility of these chemicals in vegetable oil or *n*-octanol and water along with information on the blood levels of lipids and water (Poulin and Krishnan, 1995, 1996b; Poulin *et al.*, 1999). Although these algorithms accurately predicted the rat $P_{b:a}$ of alcohols, esters, and ketones, a large discrepancy appeared when these algorithms were used to predict the rat $P_{b:a}$ of relatively lipophilic VOCs such as alkanes, haloalkanes, and aromatic hydrocarbons. This discrepancy was attributed to the potential binding of these lipophilic VOCs to rat hemoglobin (Hb) (Poulin and Krishnan, 1996a). Following a detailed study of the structural features of VOCs, it was hypothesized that chemicals lacking oxygen in their molecule, having a log oil:water PC $\geq$ 1. and a geometric volume $\leq$ 300 $Å^3$, are likely to exhibit binding (Poulin *et al.*, 1999). Béliveau and Krishnan (1999) experimentally verified this hypothesis by comparing the $P_{b:a}$ to the partition coefficient determined using a matrix of *n*-octanol and water, equivalent to that contained in whole blood. A discrepancy due to the binding of the VOCs to the blood proteins was observed, as anticipated, in the case of four chemicals (chloroform [CF], bromoform [BF], chlorobenzene [CB], and ethylbenzene [EB]). These studies also showed that rat Hb contributed significantly to the $P_{b:a}$ of three out of these four VOCs. For CB, CF,

and BF, Hb binding contributed up to 96% of the value of $P_{b:a}$, but in the case of EB, it contributed only to 60%, indicating the possibility that some chemicals might bind to other proteins, such as albumin, present in rat blood.

In vial equilibration studies designed to determine $P_{b:a}$ of VOCs, a single concentration of chemical in the vial has routinely been used (Sato and Nakajima, 1979; Fiserova-Bergerova, 1983; Perbellini *et al.*, 1985; Fiserova-Bergerova and Diaz, 1986; Johanson and Dynesius, 1988; Gargas *et al.*, 1989; Kaneko *et al.*, 1994; Tardif *et al.*, 1997). Unfortunately, this approach does not facilitate the identification of the occurrence and possible consequences of blood protein binding. For example, a decrease in $P_{b:a}$ at high exposure concentrations can occur due to the increase in unbound chemical concentration resulting from the saturation of binding sites. Dose-dependency of $P_{b:a}$, or the linear range of exposure concentrations where the $P_{b:a}$ is unaffected, would depend mainly on the association constant (K_a) of the chemical for the blood proteins and the number of binding sites (n) on the rat blood proteins. Although the K_a for the binding of several drugs and VOCs to many human blood proteins has been experimentally determined (Goldstein, 1949; Meyer and Guttman, 1968; Vallner, 1977; Paxton, 1985; Hinderling, 1997), the experimental determination of the K_a for VOC binding to rat blood proteins has not yet been performed. Poulin and Krishnan (1996a) previously estimated the K_a for several VOCs, using the solubility-based predictions of the $P_{b:a}$ and the experimentally determined $P_{b:a}$ found in the literature. The K_a was therefore estimated using only one vial exposure concentration, whereas robust K_a estimation methods require the use of data from

a wide range of exposure concentrations (Goldstein 1949; Bridges and Wilson, 1976; Lindup, 1987). Further, the methodology used by Poulin and Krishnan (1996a) did not enable the estimation of the number of binding sites (n) possibly an important parameter, given that chemicals bind to a multitude of sites. The average K_a value derived using this theoretical method is 1.93 mM^{-1}, which is within the range of association constants for the binding of several drugs to human blood proteins (Lindup, 1987). The generation of data on the concentration-dependancy of PCs using vial equilibration studies with blood and oil+water mixtures might provide a scientifically-sound basis for estimating K_a and n for VOCs.

The objectives of this study were therefore: i) to determine the dose-dependency of $P_{b:a}$ of four VOCs (BF, CF, CB, and EB) known to bind to rat blood proteins, and ii) to derive K_a and n values for the binding of these chemicals to rat blood proteins.

MATERIALS AND METHODS

Chemicals

BF (99+%), CF (99.9%), CB (99%), and EB (99%) were purchased from Aldrich Chemical Co. (Oakville, Ontario, Canada). *n*-Octanol (99.99%) was procured from Fischer Scientific Ltd (Nepean, Ontario, Canada).

Animals

Male Fischer 344 rats (190-250g) were purchased from Charles River Canada, Inc. (St-Constant, Quebec, Canada). The rats were housed in groups of three in stainless steel cages and maintained on a 12-hour light/dark cycle under controlled temperature and relative humidity. Food and water were provided *ad libitum* throughout an acclimation period of 7 days. Rats were then sacrificed following anesthesia with CO_2, and heparinized blood from the animals was collected and pooled.

Experimental set-up

I. Determination of the concentration dependency of blood:air PCs of VOCs

The concentration dependency of the PCs of BF, CF, CB, and EB was determined by vial equilibration technique as described by Gargas *et al.* (1989), and modified

by Tardif *et al.* (1997). The experimental procedure for determining the PCs involved: i) introducing 0.11 to 20.2 μmol of EB, 0.11 to 24.6 μmol of CB, 0.11 to 11.4 μmol of BF, and 0.11 to 186.6 μmol of CF into sealed glass vials (22 ml) containing the matrices (0.1 to 0.5 ml of blood, or a mixture of *n*-octanol and water) and ii) quantifying the headspace concentrations of the chemicals at 1 hr following their introduction into the vials. The matrices either consisted of pooled rat blood obtained as described above, or a mixture of *n*-octanol and water. The volumes of oil (2 μl) and water (842 μl) used in these experiments correspond to the volumes of neutral lipid-like components and water-like components reported to be present in 1 ml rat blood (Poulin and Krishnan, 1996b). Maximal level of the total quantity injected varied from chemical to chemical according to their saturation levels in air. The vials were placed in a shaker-incubator and maintained at 37°C throughout the experimental procedure.

Chemical analysis

The concentrations of chemicals in the headspace of glass vials were determined by injecting 1 ml of the headspace with a TEKMAR® headspace Autosampler 7000 into a gas chromatograph (Hewlett Packard 5890) equipped with a flame ionization detector. Separation and quantification of VOCs were performed using a HP-1 column, 30 m I.D., 0.53 mm, 2.65 μm film thickness with helium as the carrier gas. Detector temperature was 250°C and column temperature was 55°C. Oven temperature settings (115-150°C) were adjusted to keep adequate peak resolution for the wide range of concentrations measured.

Data analysis

Using the headspace concentrations of the chemicals determined at equilibrium, PCs were calculated as follows (Gargas *et al.*, 1989):

$$PC = \frac{(C_r * V_r) - \left[C_s * (V_r - V_s)\right]}{C_s * V_s} \qquad [1]$$

where PC = $P_{b:a}$ or *n*-octanol+water:air PC ($P_{o+w:a}$)

C_r = VOC concentration in the empty, reference vial

C_s = VOC concentration in the headspace of the vial containing the matrix

V_r = Volume of the reference vial, and

V_s = Volume of the sample

Statistics

The numerical values of $P_{b:a}$ and $P_{o+w:a}$ for each chemical were compared using ANOVA. Post Hoc comparison was performed using Scheffé's test. Significance was determined at p value of 0.05.

II. Determination of n and K_a for VOC binding to rat blood proteins

The n and K_a values for VOC binding to blood proteins were estimated using a double-reciprocal plot of binding data, based on the equation (Lindup, 1987):

$$\frac{1}{r} = \frac{1}{n \cdot K_a} \cdot \frac{1}{C_u} + \frac{1}{n} \qquad [2]$$

where

r = molar ratio of bound chemical calculated as $\frac{C_b}{C_{pu} + C_b}$, C_{pu} being the concentration of free protein (C_{pu} + C_b = 2.34 mM for Hb or 8.13 mM for total blood protein) (Long, 1961) and C_b being concentration of bound chemical calculated as $C_b = C_t - C_u$

C_t = concentration of total VOC in blood sample calculated as the product of C_{hs} and the experimentally determined $P_{b:a}$

C_u = unbound concentration of chemical calculated as $C_u = C_{hs} \cdot P_{o+w:a}$

C_{hs} = concentration of VOC in headspace of vial, and

$P_{o+w:a}$ = experimentally determined *n*-octanol+water:air PC

The plot of 1/r vs $1/C_u$ yields a straight line with a slope of $1/nK_a$ and an intercept of 1/n. Binding data were also graphed in a semi-log plot to verify that the half saturation point had been reached (Klotz, 1982, 1983, 1985; Faguet, 1986; Lindup, 1987). Using the data on C_u, C_b, and C_t, the K_a and n values for VOC binding to blood proteins were estimated.

RESULTS

The $P_{b:a}$, $P_{o+w:a}$, as well as C_u values for BF, CF, CB, and EB as a function of the amount injected into sealed vials are presented in Tables 1-4. For all the four VOCs, the numerical values of $P_{o+w:a}$ were significantly lower than that of $P_{b:a}$ determined in all experiments, confirming the presence of protein binding for these VOCs in rat blood. With increasing amount injected, the $P_{b:a}$, and not $P_{o+w:a}$, showed a tendency to decrease. There is a statistically significant decrease in the $P_{b:a}$ at the higher amounts injected for EB, CB, and CF, whereas BF shows a qualitative, but not significant decrease in the $P_{b:a}$. The ratio of the highest to the lowest $P_{b:a}$ determined in these experiments varied from 1.4 to 3.5 for the four VOCs. Even though somewhat variable the average of the results for $P_{o+w:a}$ of each chemical was used to estimate its C_u. Using the C_u and r values, double-reciprocal plots of the binding data were constructed for the four VOCs using total rat blood proteins (Figure 1), or rat Hb (Figure 2) as the binding protein. These plots were then used to derive the K_a and n values (Tables 5 and 6). As seen in Tables 5 and 6, the estimated n values vary from 1 to 4, whereas the K_a values vary from 0.183 to 2.748 mM^{-1}.

DISCUSSION

Previous studies have suggested that consideration of the role of possible protein binding is important to adequately predict the $P_{b:a}$ of lipophilic VOCs such as BF, CB, CF and EB (Poulin and Krishnan, 1996a; Poulin *et al.*, 1999; Béliveau and Krishnan, 1999). The limited presence of binding sites on proteins implies that, at a certain exposure concentration (determined by the K_a and n), these sites will become saturated with the ligand. Beyond this point, the bound concentration of chemicals will be equal nC_p, whereas unbound chemical concentration will continue to increase as a function of exposure concentration. As exposure concentration increases, C_b becomes negligible relative to C_u. Under such conditions, $P_{b:a}$ approximates $P_{o+w:a}$. The results of the present study show a decrease (38% for EB, 46% for CB, 28% for BF, and 35% for CF) in $P_{b:a}$ as the amount of chemical injected within the vial increased. Although this consequence of rat blood protein binding of lipophilic VOCs has previously been suggested (Poulin and Krishnan, 1996a; Kumarathasan *et al.*, 1998), the present study provides the first experimental evidence of the occurrence of such a process. The dose-dependency seen in rat blood is not observed when the $P_{o+w:a}$ is evaluated at different exposure concentrations, suggesting that solubility within *n*-octanol and water is the only mechanism driving this PC. The observed dose-dependency in rat blood would depend on K_a and n, which are likely to be chemical-specific.

K_a values for VOC binding to Hb have been previously derived based on the discrepancy between experimental and solubility-based predictions of $P_{b:a}$ (Poulin

and Krishnan, 1996a). Actually, this theoretical approach generated nK_a values and not K_a values, and therefore the number of binding sites could not be determined. In addition, $P_{b:a}$ found in the literature are usually determined using only one exposure concentration in vial equilibration studies. Rigorous K_a determination requires the use of a wide range exposure concentrations (Goldstein, 1949; Meyer and Guttman, 1968; Bridges and Wilson, 1976; Lindup, 1987). A better approach for estimating the K_a of VOCs that display binding would be to determine the PCs of VOCs in rat blood and in an equivalent lipid+water mixture at increasing exposure concentrations. Although the $P_{b:a}$ decreases at higher exposure concentrations due to the saturation of available binding sites on the binding proteins, the corresponding $P_{o+w:a}$, representing the solubility of the VOC, should not vary. Using the lipid+water solubility, which can be used to estimate the unbound fraction of the VOC in blood, and the experimental $P_{b:a}$, which can be used to calculate the total amount of VOC present in the blood sample, we can calculate the bound concentration of chemical (Chiou and Hsiao, 1974), as done in the present study.

With the PCs (blood:air and *n*-octanol+water:air) of the four chemicals used in this study at multiple exposure concentrations, we were able to characterize the binding of these chemicals to either rat total blood proteins or Hb. Evidence suggests that although Hb contributes significantly to the $P_{b:a}$ other proteins are also, but to a lesser extent, ligands for VOCs (Béliveau and Krishnan, 1999). The most common form of representing binding data has traditionally been the Scatchard plot, where r/C_u is plotted against r. Even though this method has been

widely used, the statistical merit of this analysis has been questioned (Wilkinson, 1983). An alternative graphical method that is often used is the double reciprocal plot, similar to Lineweaver-Burk plot commonly used in enzyme kinetics studies. When this graphical analysis is combined to semi-log plot analysis to verify the attainment of the half-saturation point during the experiments as been done in the present study, more accurate estimations are made (Klotz, 1982).

This study represents the first effort to experimentally evaluate the parameters of VOC binding to rat blood proteins. In this case, whole blood was used, and not a pure protein solution. Nevertheless, using either total protein or Hb concentration, an estimation of the general non-specific binding parameters could be made. The number of estimated binding sites ranges from one for CB to five for BF, with CB having the highest binding affinity. BF, the most lipophilic VOC (log oil:water PC = 2.40) used in this study also has the highest number of binding sites, but not necessarily the highest affinity for the sites. When considering the binding as a function of hemoglobin as the binding protein, only BF seems to have more than 1 binding site, and again CB has the highest affinity for the binding site. The nK_a values estimated in this study (CB = 3.902 mM^{-1} and CF = 2.087 mM^{-1}) compare well with the values (CB = 3.279 mM^{-1} and CF = 2.383 mM^{-1}) previously derived by Poulin and Krishnan (1996a) based on theoretical considerations. The slightly lower K_a values obtained in this study can be explained by the generally slightly lower $P_{b:a}$ at lower exposure concentrations determined in this study compared to Gargas *et al.* (1989), or simply by the fact that the PCs in this study were determined over a range of exposure concentrations.

The demonstration of the dose-dependency of $P_{b:a}$ *in vitro* in rat blood may have important implications for the development of PBPK models. It illustrates the importance of determining the range of exposure concentrations where the physicochemical parameters (particularly the $P_{b:a}$) are not altered, especially in the case of animal bioassays, where high exposure concentrations are often used. Based on the results in this study, there are no significant changes in the $P_{b:a}$ at *in vitro* exposures below 5465 ppm for BF, CB, CF, and EB. Therefore, when modeling inhalation exposures for up to 5000 ppm, the dose dependent nature of the $P_{b:a}$ need not be considered for these chemicals. However, this concentration will vary according to the K_a and n of the chemical, and therefore lower or higher threshold values for dose dependency of $P_{b:a}$ are possible for other chemical. The knowledge of the dose-dependency of $P_{b:a}$ is essential to ensure their appropriate use in PBPK models.

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REFERENCES

Béliveau, M., and Krishnan, K. 2000. Determinants of blood protein binding of volatile organic chemicals. *Toxicol. Lett.* (Submitted).

Bridges, J. W., and Wilson, A. G. E. 1976. Drug-serum protein interactions and their biological significance. In *Progress in Drug Metabolism,* eds. J. W. Bridges and L. F. Chasseaud, pp. 193-247. Chichester: Wiley.

Chiou, W.L., and Hsiao, J.-H. 1974. A new simple approach to study the protein binding of volatile and gaseous I. Fluorocarbon aerosol propellants, halothane and cyclopropane. *Res. Comm. Chem. Pathol. Pharmacol.* **8**: 273-287.

Faguet, G.B. 1986. Number of receptor sites from Scatchard and Klotz graph: complementary approaches. *J. Cell. Biochem.* **31**: 243-250.

Fiserova-Bergerova, V. 1983. Gases and their solubility: a review of fundamentals. In *Modeling of Inhalation exposure to vapors: uptake, distribution and elimination,* ed. V. Fiserova-Bergerova, pp. 3-28. Boca Raton: CRC Press.

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

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

Goldstein, A. 1949. The interactions of drugs and plasma proteins. *Pharmacol. Rev.* **1**: 102-165.

Hinderling, P.H. 1997. Red blood cells: a neglected compartment in pharmacokinetics and pharmacodynamics. *Pharmacol Rev.* **49**: 279-295.

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

Kaneko, T., Wang, P.Y., and Sato, A. 1994. Partition coefficients of some acetate esters and alcohols in water, blood, olive oil, and rat tissues. *Occup. Environ. Med.* **51**: 68-72.

Klotz, I.M. 1982. Numbers of receptor sites from Scatchard graphs: facts and fantasies. *Science* **217**: 1247-1249.

Klotz, I.M. 1983. Ligand-receptor interaction: what we can and cannot learn from binding measurements. *Trends Pharmacol. Sci.* **4**: 253-255.

Klotz, I.M. 1985. Estimation of number of receptor sites: a misconception. *Biopharm. Drug Dispos.* **6**: 105-106.

Krishnan, K., and Andersen, M. E. 1994. Physiologically based pharmacokinetic modeling in toxicology. In *Principles and Methods of Toxicology,* ed. W. A. Hayes, pp. 149-188. New York: Raven Press, Ltd.

Kumarathasan, P., Otson, R., and Chu, I. 1998. Application of an automated HS-GC method in partition coefficient determination for xylenes and ethylbenzene in rat tissues. *Chemosphere* **37**: 159-178.

Lindup, W.E. 1987. Plasma protein binding of drugs - some basic and clinical aspects. *Prog. Drug Metab.* **10**: 141-185.

Long, C. 1961. *Biochemist's Handbook,* London: E & F Spon Ltd.

Meyer, M.C., and Guttman, D.E. 1968. The binding of drugs by plasma proteins. *J. Pharm. Sci.* **57**: 895-918.

Paxton, J.W. 1985. Drug binding to plasma proteins. *New Zeal. Med. J.* **98**: 245-248.

Perbellini, L., Brugnone, F., Caretta, D., and Maranelli, G. 1985. Partition coefficients of some industrial aliphatic hydrocarbons (C5-C7) in blood and human tissues. *Br. J. Ind. Med.* **42**: 162-167.

Poulin, P., Béliveau, M., and Krishnan, K. 1999. Mechanistic animal-replacement approaches for predicting pharmacokinetics of organic chemicals. In *Toxicity*

Assessment Alternatives: Methods, Issues, Opportunities, eds. H. Salem and S. A. Katz, pp. 115-139. Totowa: Humana Press Inc.

Poulin, P., and 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., and Krishnan, K. 1996a. 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.

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

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

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.

Vallner, J.J. 1977. Binding of drugs by albumin and plasma protein. *J. Pharm. Sci.* **66**: 447-465.

Wilkinson, G.R. 1983. Plasma and tissue binding considerations in drug disposition. *Drug Metab. Rev.* **14**: 427-465.

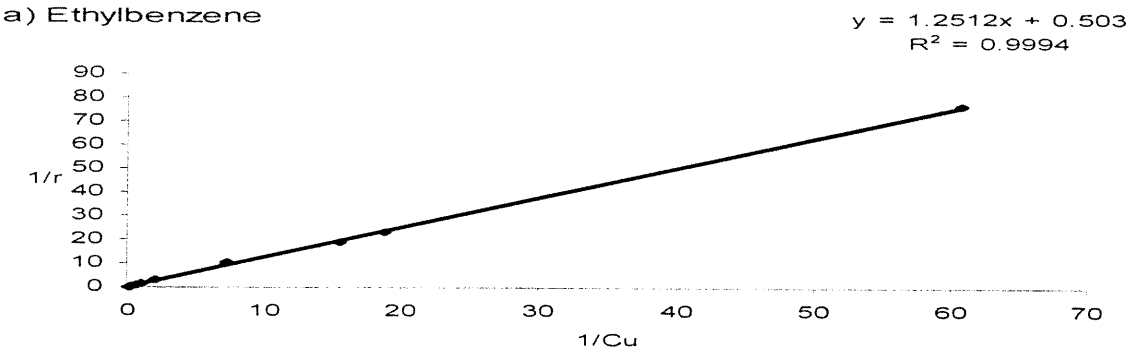
FIGURE LEGENDS

FIGURE 1: Klotz plot of the reciprocal of the molar ratio of bound VOC (1/r) versus the reciprocal of unbound concentration ($1/C_u$) using total blood protein concentration for: a) Ethylbenzene, b) Chlorobenzene, c) Bromoform, and d) Chloroform.

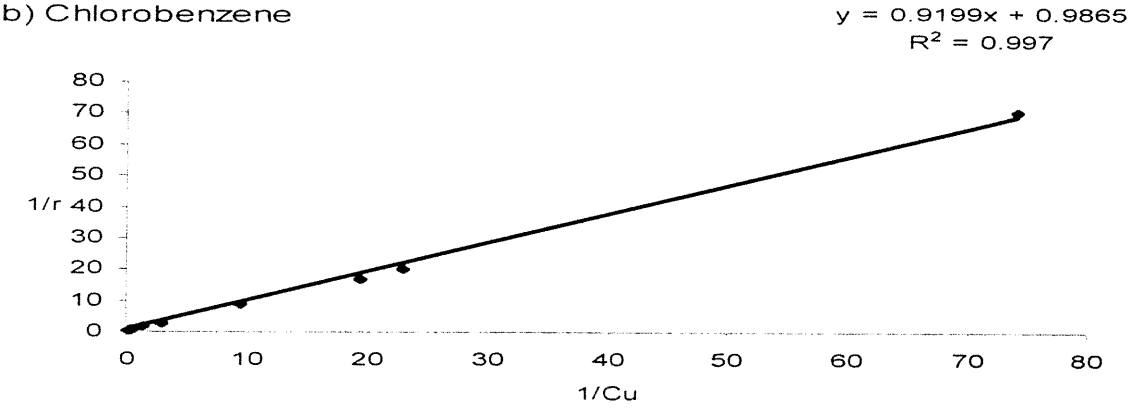
FIGURE 2: Klotz plot of the reciprocal of the molar ratio of bound drug (1/r) versus the reciprocal of unbound concentration ($1/C_u$) using hemoglobin concentration for: a) Ethylbenzene, b) Chlorobenzene, c) Bromoform, and d) Chloroform.

FIGURE 1

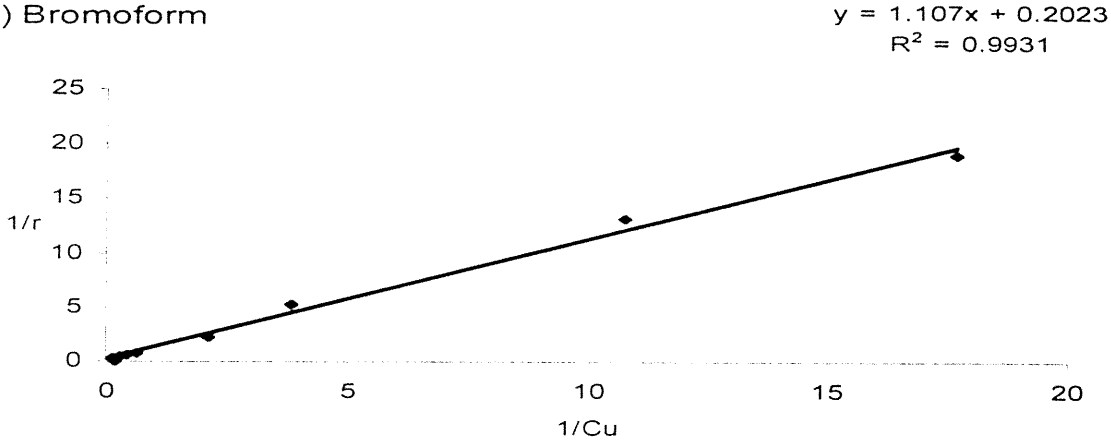
a) Ethylbenzene



b) Chlorobenzene



c) Bromoform



d) Chloroform

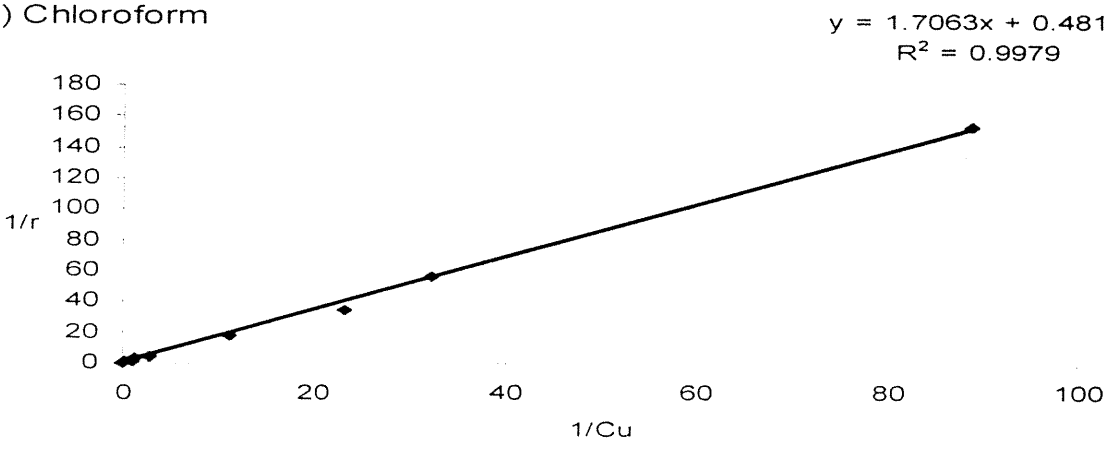
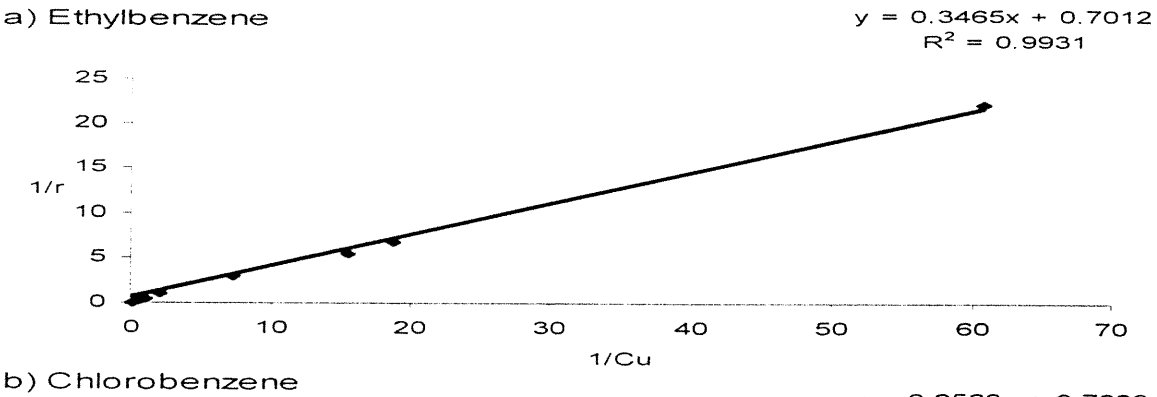


FIGURE 2

a) Ethylbenzene



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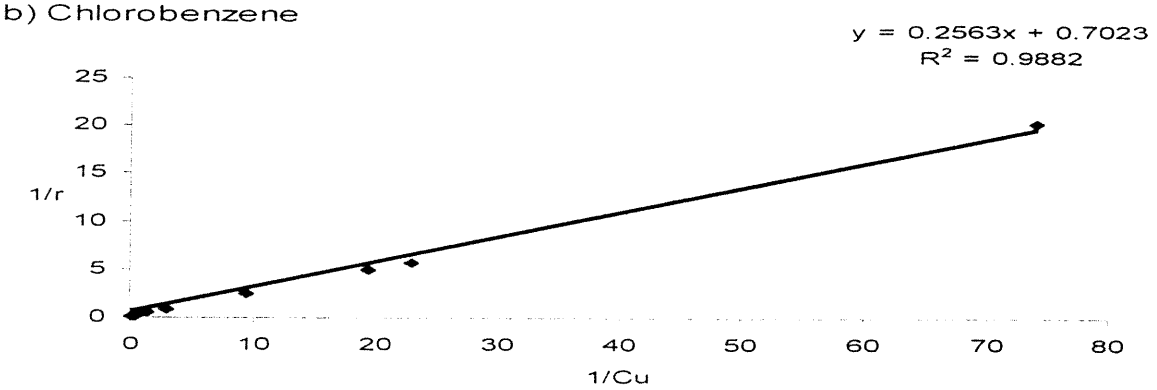
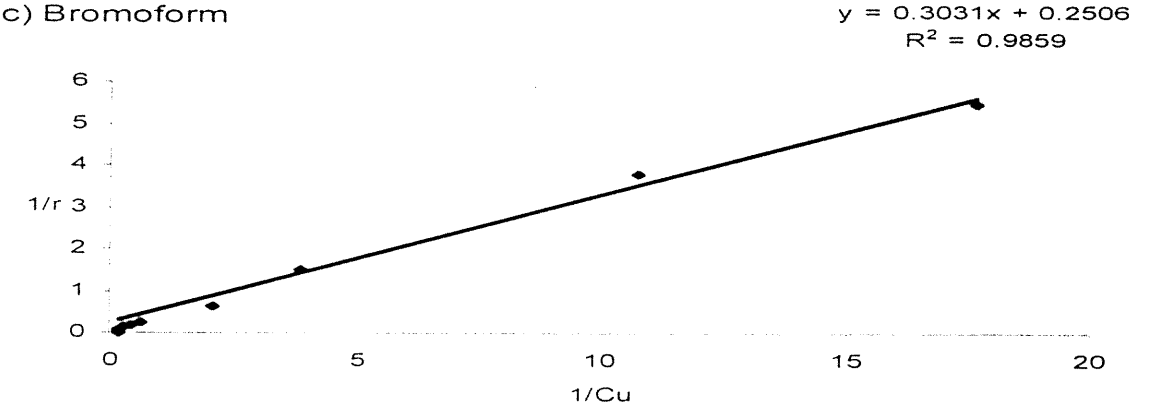


c) Bromoform



d) Chloroform

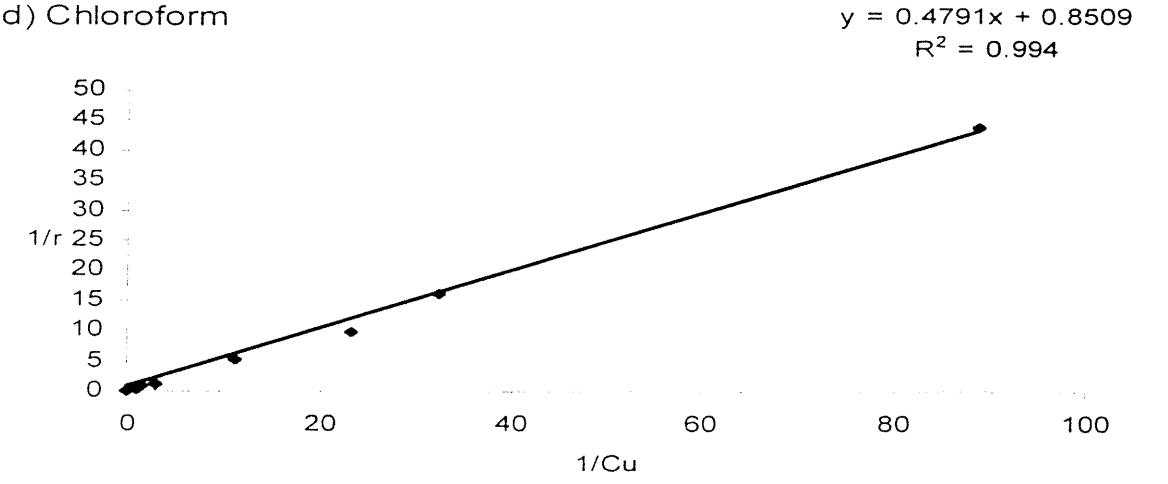


TABLE 1: Blood:air partition coefficient ($P_{b:a}$), *n*-octanol+water:air partition coefficient ($P_{o+w:a}$), and unbound concentration (C_u) of bromoform as a function of the amount of this chemical injected into sealed vials.

Chemical (μmol) injected	$P_{b:a}$ [a]	$P_{o+w:a}$ [a]	C_u (mM) [b]
9.73	129±2.4	24±5.8	5.87
5.72	130±2.2	15±0.8	3.44
4.01	150±5.6	22±1.8	2.28
2.86	155±8.8	16±0.5	1.61
0.90	180±17	27±0.5	0.48
0.45	145±12	18±1.7	0.26
0.27	161±5.2	19±3.4	0.09
0.11	180±12	19±1.3	0.06

[a] : values presented as mean±S.E. (n=3-4)

[b] : C_u was calculated as the product of measured headspace concentration and $P_{o+w:a}$

TABLE 2: Blood:air partition coefficient ($P_{b:a}$), *n*-octanol+water:air partition coefficient ($P_{o+w:a}$), and unbound concentration (C_u) of chlorobenzene as a function of the amount of this chemical injected into sealed vials.

Chemical (μmol) injected	$P_{b:a}$[a,b]	$P_{o+w:a}$[a]	C_u(mM)[c]
24.6	31±1.3*	6±0.18	4.35
19.7	37±1.5*	9±0.12	3.23
14.8	32±0.8*	7±0.06	2.56
9.83	33±1.9*	6±0.29	1.70
4.93	41±4.2*	6±0.74	0.78
2.46	54±3.8	7±0.37	0.34
0.90	59±3.2	5±1.14	0.11
0.45	62±2.5	4±0.18	0.05
0.27	62±2.8	8±2.35	0.04
0.11	57±2.5	6±0.07	0.01

[a] : values represent mean±S.E. (n=3-4)

[b] : * denotes statistical significance compared to the lowest injected quantity.

[c] : C_u was calculated as the product of measured headspace concentration and $P_{o+w:a}$

TABLE 3: Blood:air partition coefficient ($P_{b:a}$), *n*-octanol+water:air partition coefficient ($P_{o+w:a}$), and unbound concentration (C_u) of chloroform as a function of the amount of this chemical injected into sealed vials.

Chemical (μmol) injected	$P_{b:a}$ [a,b]	$P_{o+w:a}$ [a,b]	C_u (mM)[c]
186	6±0.32*	5±1.40	23.94
62.2	6±0.47*	5±0.17	7.93
37.3	9±0.42*	3±0.25	4.60
9.32	19±1.5	2±0.27	1.02
6.20	15±1.6	2±0.71	0.73
3.11	21±1.2	4±1.24	0.35
0.90	18±0.6	3±0.37	0.09
0.45	20±1.2	3±0.27	0.04
0.27	17±1.1	3±0.75	0.03
0.11	17±0.4	2±0.30	0.01

[a] : values represent mean±S.E. (n=3-5)

[b] : * denotes statistical significance compared to lowest injected quantity.

[c] : C_u was calculated as the product of measured headspace concentration and $P_{o+w:a}$

TABLE 4: Blood:air partition coefficient ($P_{b:a}$), *n*-octanol+water:air partition coefficient ($P_{o+w:a}$), and unbound concentration (C_u) for ethylbenzene as a function of the amount of this chemical injected into sealed vials.

Chemical (μmol) injected	$P_{b:a}$ [a,b]	$P_{o+w:a}$ [a]	C_u (mM) [c]
17.0	31±0.5*	8±0.46	3.09
8.47	37±0.5	5±1.32	1.41
4.25	43±1.0	5±0.33	0.94
2.12	41±0.7	6±0.96	0.47
0.90	45±2.1	7±0.66	0.14
0.45	51±4.4	5±0.47	0.06
0.27	51±1.3	7±1.57	0.05
0.11	50±4.2	6±0.97	0.02

[a] : values represent mean±S.E. (n=3-4)

[b] : * denotes statistical significance compared to the lowest injected quantity.

[c] : C_u was calculated as the product of measured headspace concentration and $P_{o+w:a}$

TABLE 5: Rat blood protein (total fraction) association constant (K_a) and number of binding sites (n), as derived from the binding data, for the four VOCs used in this study

Chemicals	K_a (mM^{-1})	*n*	nK_a (mM^{-1})
Bromoform	0.183	4.94	0.904
Chlorobenzene	1.076	1.01	1.087
Chloroform	0.282	2.08	0.587
Ethylbenzene	0.402	1.99	0.800

TABLE 6: Rat blood protein (hemoglobin fraction) association constant (K_a) and number of binding sites (n), as derived from the binding data, for the four VOCs used in this study

Chemicals	K_a (mM^{-1})	*n*	nK_a (mM^{-1})
Bromoform	0.825	4	3.300
Chlorobenzene	2.748	1.42	3.902
Chloroform	1.769	1.18	2.087
Ethylbenzene	2.018	1.43	2.886

CHAPITRE CINQUIÈME :

5 – DISCUSSION GÉNÉRALE

La modélisation PCBP permet la prédiction de la cinétique d'une substance dans une espèce à l'aide de paramètres physiologiques, biochimiques, et physico-chimiques. Des algorithmes mécanistes peuvent être utilisés pour prédire la valeur des paramètres physicochimiques, comme le P_b, en utilisant des informations sur la solubilité des substances dans les composants tissulaires tel que les lipides et l'eau. Toutefois, les valeurs de P_b estimées chez le rat pour les SOV relativement lipophiles (p. ex., alcanes, haloalcanes, et aromatiques) sont inférieures aux valeurs déterminées expérimentalement. Bien que cette discordance entre les valeurs estimées et expérimentales ait été attribuée à la présence de liaisons entre les SOV et les protéines du sang de rat, une évaluation expérimentale de la liaison aux protéines sanguines n'a jamais été effectuée. Le présent projet de recherche avait pour objectif de combler cette lacune.

L'hypothèse de la liaison aux protéines sanguines du rat émise par Poulin et Krishnan (1996a) était basée sur l'existence de certaines structures ("cavités") hydrophobes sur les protéines sanguines humaines, comme l'Hb (Featherstone et Schoenborn, 1964). Ces structures peuvent lier faiblement de nombreux SOV et anesthésiques (Featherstone *et al.*, 1961; Schoenborn, 1968, 1976; Brown *et al.*, 1976; Eckenhoff et Johansson, 1997). Puisque la structure tertiaire des protéines du sang est relativement semblable entre les espèces (Lindup, 1987), ces structures sont fort probablement présentes chez le rat également. De plus, les protéines du sang de rat contiennent généralement plus d'acides aminés hydrophobes (Lindup, 1987), ce qui laisse prévoir une plus grande importance de la liaison chez le rat. Il est reconnu qu'il existe un lien étroit entre la structure des substances liées et celle de leurs sites de liaison sur les protéines ("clé et serrure")

(Franks, 1985; Curry, 1990). Le nombre de site sur l'Hb humaine étant limité (Schoenborn, 1976), il est raisonnable de penser que certaines caractéristiques sont communes à toutes les substances liantes.

Ainsi dans l'Article I, certaines caractéristiques moléculaires communes aux SOV pressentis comme étant des ligands de l'Hb ont été déterminées. Suite à ceci, la présence ou l'absence de liaison à été confirmée expérimentalement pour certains SOV sélectionnés à partir de ces déterminants et la fraction protéinique impliquée a été déterminée pour des SOV montrant de la liaison aux protéines sanguines (Article II). Finalement, dans l'Article III, la valeur de K_a et n a été déterminée, caractérisant plus en détail les paramètres de cette liaison.

Dans l'Article III, il a été démontré que pour les SOV utilisés dans cette étude, la liaison protéinique avait un impact seulement à de hautes concentrations d'exposition, la plus faible concentration ayant un effet étant 5465 ppm pour le chlorobenzène. Au cours de plusieurs études portant sur la toxicité (cancérogénicité, toxicité hépatique, toxicité développementale) de certains SOV chez le rat, les animaux sont exposés à de hautes concentrations de substance variant de 8000 à 800 000 ppm (Tableau 1), dépendant de la volatilité de la substance. Lorsque la prédiction de la cinétique des SOV chez le rat par modélisation PCBP à de telles concentrations d'exposition est nécessaire, la liaison potentielle de ces substances aux protéines sanguines et la variation de la valeur du P_b doivent être considérées. En se basant sur les résultats obtenus dans cette étude, il est possible de développer une démarche qui considère la liaison lors de la modélisation PCBP (Figure 1). Lorsque la presence de liaison entre le SOV et les protéines sanguines est soupçonnée suite à une interrogation sur ies

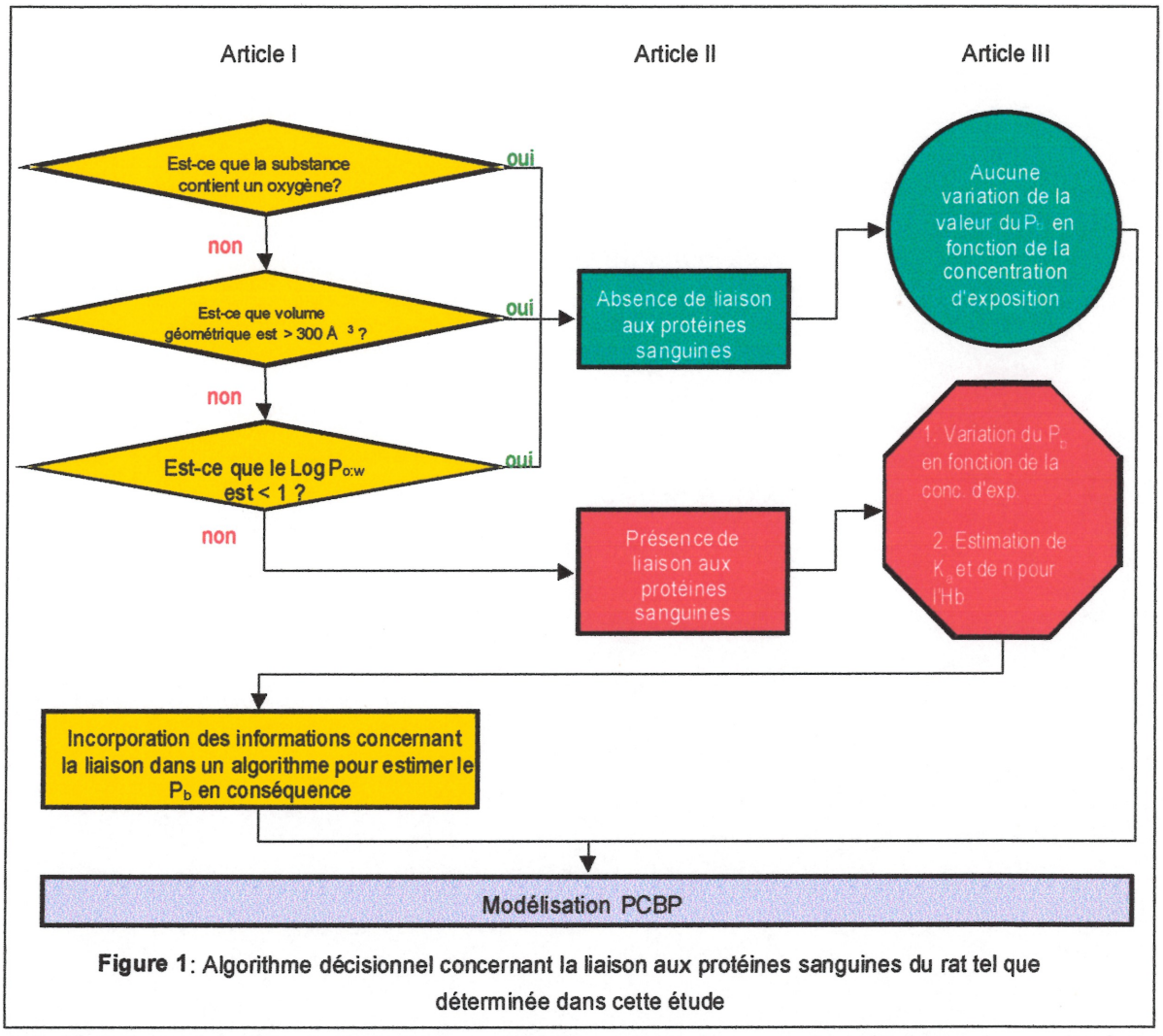


Figure 1: Algorithme décisionnel concernant la liaison aux protéines sanguines du rat tel que déterminée dans cette étude

caratéristiques moléculaires de la substance, la présence de la liaison, le seuil où il y aura baisse de la valeur du P_b, et la valeur de K_a et de n, peuvent être déterminés expérimentalement. Ces résultats peuvent ensuite être incorporés dans un algorithme (développé dans l'Article I) qui, lorsque qu'intégré dans un modèle PCBP, estime le P_b en fonction de la liaison aux protéines sanguines du rat. Comme mentionné dans l'article II, si la saturation des sites de liaison sur l'Hb du rat et la baisse du P_b à de hautes concentrations d'exposition ne sont pas considérées, la liaison pourrait avoir un impact majeur sur les extrapolations haute dose-faible dose. En effet, à de hautes doses, le P_b unique estimé *in vitro* à de faible concentrations (Gargas *et al.*, 1989) serait plus élevé que le P_b apparent (qui

est influencé par la saturation des sites de liaison), ce qui modifierait la cinétique prédite de la substance dans l'organisme à ces concentrations d'exposition.

En plus de son impact sur l'extrapolation haute dose-faible dose intra-espèce, l'effet de la liaison protéinique sur le P_b chez le rat pourrait avoir un impact sur les extrapolations inter-espèces. Par exemple, lors de l'extrapolation animal-humain utilisant la modélisation PCBP, il est important de considérer l'absence de liaison chez l'humain. Bien que cette hypothèse d'absence de liaison soit basée sur l'observation que la valeur des P_b expérimentaux chez l'humain peut être prédite par l'equation 14, des études de tubes à l'équilibre similaires à celles décrites dans l'article II seraient nécessaires afin de confirmer l'absence de liaison dans le sang humain. Ainsi, lors de la modélisation de la cinétique à haute dose chez le rat, un P_b variable en fonction de la dose serait utilisé alors que lors d'une modélisation de la cinétique chez l'humain, l'utilisation d'un P_b invariable serait suffisante.

En se basant sur les résultats de cette étude et avec la réalisation de certaines expérimentations futures, il est possible d'utiliser de façon plus judicieuse le P_b dans les modèles PCBP chez le rat ou l'humain. Les outils développés pour les SOV dans le cadre de cette étude peuvent servir à mieux comprendre certains mecanismes tel que la solubilité dans l'eau et les lipides, ainsi que la liaison aux protéines sanguines, qui régissent le partage entre le sang et l'air chez le rat.

Tableau 1: Concentrations d'exposition par inhalation de certains SOV lors d'études de toxicité chez le rat

Substance	Concentration (ppm)	Source
Bromochlorodifloromethane	50 000	Wickramaratne et al., 1988
1,3-Butadiene	7760	Dahl, *et al.*, 1991
	8000	Owen et Glaister, 1990 Owen *et al.*, 1987
1-Chloro-2,2,2-trifluoroethane	50 000	Ellis *et al.*, 1995
t-1,2-Dichloroethylene	12 000	Hurtt *et al.*, 1993
1,1-Difluoroethylene	82 000	Jaeger *et al.*, 1975
Difluoromethane	50 000	Ellis *et al.*, 1996
Ethylchloride	10 000	Landry *et al.*, 1982
1,1,1,1,2,2-Pentafluoroethane	800 000	Kawano *et al.*, 1995
Pentane	10 000	Hurtt et Kennedy, 1999
1,1,1,2-Tetrafluoroethane	50 000	Collins *et al.*, 1995
1,1,1-Trifluoroethane	40 000	Brock *et al.*, 1996
Vinyl chloride	46 500	Jaeger *et al.*, 1975

CHAPITRE SIXIÈME :

6 - BIBLIOGRAPHIE

Abraham, MH., Kamlet, MJ., Taft, W., Doherty, RM., et Weathersby, PK. (1985). Solubility properties in polymers and biological media: 2. The correlation and prediction of the solubilities of non electrolytes in biological tissues and fluids. *J. Med. Chem.* 28, 865-870.

Adolph, E.F. (1949). Quantitative relations in the physiological constitutions of mammals. *Science* **109**, 579-585.

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

Andersen, M.E., Gargas, M.L., Jones, R.A., et Jenkins, L.J. (1980). Determination of the kinetic constants for metabolism of inhaled toxicants in vivo by gas uptake measurements. *Toxicol. Appl. Pharmacol.* **54**, 100-116

Arms, A. D. et Travis, C. (1988). Reference Physiological Parameters in Pharmacokinetic Modeling. Office of Health and Environmental Assessment. EPA/600/6-88/004:1-1-7.16.

Brock, W.J., Trochimowicz, H.J., Farr, C.H., Millischer, R.J., et Rusch, J.M. (1996). Acute, subchronic, and developmental toxicity and genotoxicity of 1,1,1-trifluoroethane (HFC-143a). *Fund. Appl. Toxicol.* **31**, 200-209.

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

Carfagna, M.A. et Kedderis, G.L. (1992). Isolated hepatocytes as in vitro models for the biotransformation and toxicity of chemicals in vivo. *CIIT Activities* **12**, 1-6.

Caster, W.O., Poncelet, J., Simon, A.B., et 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.

Chen, H.S.G. et Gross, J.F. (1979). Estimation of tissue to plasma partition coefficients used in physiological pharmacokinetic models. *J. Pharmacokinetics Biopharm.* **7**, 117-125.

Clewell, H.J., III et Andersen, M.E. (1987). Dose, species and route extrapolation using physiologically-based pharmacokinetic models. *Drinking Water and Health.* **8**, 159-182.

Collins, M.A., Rusch, G.M., Sato, F., Hext, P.M., et Millischer, R.J. (1995). 1,1,1,2-tetrafluoroethane: repeat exposure inhalation toxicity in the rat, developmental toxicity in the rabbit, and genotoxicity in vitro and in vivo. *Fund. Appl. Toxicol.* **25**, 271-280.

Connell, D.W., Braddock, RD., et Mani, SV. (1993). Prediction of the partition coefficient of lipophilic compounds in the air-mammal tissues systems. *Sci. Total Environ. Suppl.* Pt 2 1383-1396.

Dahl, A.R., Sun, J.D., Birnbaum, L.S., Bond, J.A., Griffith Jr., W.C. Mauderly, J.L., Muggenburg, B.A., Sabourin, P.J., et Henderson, R.F. (1991).

Toxicokinetics of inhaled 1,3-butadiene in monkeys: comparison to toxicokinetics in rats and mice. *Toxicol. Appl. Pharmacol.* **110**, 9-19.

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

Domenech, R.J., Hoffman, J.E., Noble, M.M., Saunder, K.B., Hensen, J.R., et Subijanto, S. (1969). Total and regional coronary blood flow measured by radioactive microsphere in conscious and anesthetized dogs. *Circ. Res.* **25**, 581-596.

Ellis, M.K., Naylor, J.L., Green, T., et Collins, M.A. (1995). Identification and quantification of fluorine-containing metabolites of 1-chloro-2,2,2-trofluoroethane (HCFC133A) in the rat by 19F-NMR spectroscopy. *Drug Metab. Disp.* **23**, 102-106.

Ellis, M.K., Trebilcock, R., Naylor, J.L., Tseung, K., Collins, M.A., Hext, P.M., et Green, T. (1996). The inhalation toxicology, genetic toxicology, and metabolism of difluoromethane in the rat. *Fund. Appl. Toxicol.* **31**, 243-251.

Featherstone, R.M. et Schoenborn, B.P. (1964). Protein and lipid binding of volatile anaesthetic agents. *Br. J. Anaesthesia* **36**, 150-154.

Filser, J.G. et Bolt, H.M. (1979). Pharmacokinetics of halogenated ethylenes in rats. *Arch. Toxicol.* **42**, 123-136.

Gabrielsson, J.L., Paalzow, L.K., et Nordstrom, L. (1984). A physiologically-based pharmacokinetic model for theophiline disposition in the pregnant and non-pregnant rat. *J. Pharmacokinetics Biopharm.* **12**, 149-165.

Gallo, J.M., Lam, F.C., et Perrier, D.G. (1987). Area method for the estimation of partition coefficients for physiological pharmacokinetic models. *J. Pharmacokinetics Biopharm.* **15**, 271-280.

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. (1991). Chemical-specific constants for physiologically-based pharmocokinetic models. *CIIT Activities* **11**, 2-11.

Gargas, M.L. et 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., Andersen, M.E., et 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., et 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., Seybold, P.G., et Andersen, M.E. (1988). Modeling the tissue solubilities and metabolic rate constants (Vmax) of halogenated methanes, ethanes and ethylenes. *Toxicol. Lett.* **43**, 235-256.

Haddad, S., Gad, S.C., Tardif, R., et Krishnan, K. (1995). Statistical approaches for the validation of physiologically-based pharmacokinetic (PBPK) models. *Toxicologist* **15**, 48-48.

Haddad, S., Pelekis, M., et Krishnan, K. (1996). A methodology for solving physiologically based pharmacokinetic models without the use of simulation softwares. *Toxicol. Lett.* **85**, 113-126.

Haddad, S., Withey, J., Lapare, S., Law, F., et Krishnan, K. (1998). Physiologically-based pharmacokinetic modelling of pyrene in the rat. *Environ. Toxicol. Pharmacol.* **5**, 245-255.

Hurtt, M.E., et Kennedy Jr., G.L. (1999). A limited developmental toxicity study of pentane by inhalation in the rat. *Food Chem. Toxicol.* **37**, 565-567.

Hurtt, M.E., Valentine, R., et Alvarez, L. (1993). Developmental toxicity of inhaled trans-1,2-dichloroethylene in the rat. *Fund. Appl. Toxicol.* **20**, 225-230.

Ishizaki, J., Yokogawa, K., Nakashima, E., et Ichimura, F. (1997). Relationships between the hepatic intrinsic clearance or blood cell-plasma partition coefficient in the rabbit and the lipophilicity of basic drugs. *J. Pharm. Pharmacol.* **49**, 768-772.

Jaeger, R.J., Conolly, R.B., Reynolds, E.S., et Murphy, S.D. (1975). Biochemical toxicology of unsaturated halogenated monomers. *Environ. Health Perspec.* **11**, 121-128.

Jepson, G.W., Hoover, D.K., Black, R.K., McCafferty, J.D., Mahle, D.A., et Gearhart, J.M. (1994). A partition coefficient determination method for nonvolatile chemicals in biological tissues. *Fund. Appl. Toxicol.* **22**, 519-524.

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

Kaneko, T., Wang, P.Y., et Sato, A. (1994). Partition coefficients of some acetate esters and alcohols in water, blood, olive oil, and rat tissues. *Occ. Environ. Med.* **51**, 68-72.

Kawano, T., Trochimowicz, H.J., Malinverno, G., et Rusch, G.M. (1995). Toxicological evaluation of 1,1,1,2,2-pentafluoroethane (HFC-125). *Fund. Appl. Toxicol.* **28**, 223-231.

Krishnan, K. et Andersen, M.E. (1991). Interspecies scaling in pharmacokinetics. Dans: *New Trends in Pharmacokinetics* (A. Rescigno et A.K. Thakkur, Eds.), pp. 203-226. Plenum Press, New York..

Krishnan, K. et Andersen, M.E. (1994). Physiologically-based pharmacokinetic modeling in toxicology. Dans: *Principles and Methods of Toxicology* (W.A. Hayes, Ed.), 3rd Ed., pp. 149-188. Raven Press, Ltd., New York..

Krishnan, K. et Pelekis, M. (1995). Hematotoxic interactions: occurence,mechanisms and predictability. *Toxicology* **105**, 355-364.

Krishnan, K., Andersen, M.E., Clewell, H.J., III, et Yang, R.S.H. (1994). Physiologically-based pharmacokinetic modeling of chemical mixtures. Dans: *Toxicology of Chemical Mixtures* (R.S.A., Yang, Ed.), pp. 399-437. Academic Press, New York..

Kumarathasan, P., Otson, R., et Chu, I. (1998). Application of an automated HS-GC method in partition coefficient determination for xylenes and ethylbenzene in rat tissues. *Chemosphere* **37**, 159-178.

Lam, C.W., Galen, T.J., Boyd, J.F., et Pierson, D.L. (1990). Mechanism of transport and distribution of organic solvents in blood. *Toxicol. Appl. Pharmacol.* **104**, 117-129.

Lam, G., Chen, M.L., et Chiou, W.L. (1982). Determination of tissue: blood partition coefficients in physiologically-based pharmacokinetic models. *J. Pharmaceutical Sci.* **71**, 454-456.

Landry, T.D., Ayres, J.A., Johnson, K.A., et Wall, J.M. (1982). Ethyl chloride: a two-week inhalation toxicity study and effects on liver non-protein sulfhydryl concentrations. *Fund. Appl. Toxicol.* **2**, 230-234.

Law, F.C.P., Abeini, S., et Kennedy, C.J. (1991). A biologically-based toxicokinetic model for pyrene in rainbow trout. *Toxicol. Appl. Pharmacol.* **110**, 390-402.

Lin, J.H., Hayashi, M., Awazu, S., et Hanano, M. (1978). Correlation between in vitro and in vivo drug metabolism rate: Oxidation of ethoxybenzamide in rat. *J. Pharmacokinetics Biopharm.* **6**, 327-337.

Lin, J.H., Sugiyama, Y., Awazu, S., et Hanano, M. (1982). In vitro and in vivo evaluation of the tissue to blood partition coefficients for physiological pharmacokinetic models. *J. Pharmacokinetics Biopharm.* **10**, 637-647.

Lindup, W.E. (1987). Plasma protein binding of drugs - some basic and clinical aspects. *Prog. Drug Metab.* **10**, 141-185.

Lowe, H.J. et Hagler, K. (1969). Determination of volatile organic anaesthetic in blood, gases, tissues and lipids: partition coefficients. Dans: *Gas chromatography in biology and medicine* (Ciba-Geigy Foundation Symposium), pp. 86-103. Churchill, New York.

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

Medinsky, M.A., Bechtold, W.E., Birnbaum, L.S., Chico, D.M., Gerlach, F.G., et Henderson, R.F. (1988). Uptake of vinylidene fluoride in rats simulated by a physiological model. *Fund. Appl. Toxicol.* **11**, 250-260.

Mordenti, J. et Chappell, W. (1989). The use of interspecies scaling in toxicokinetics. Dans: *Toxicokinetics and drug development* (A. Yacobi, J.P. Skelly, et V.K. Batra, Eds.), pp. 42-96. Pergamon press, New York.

Murphy, J.E., Janszen, D.B., et Gargas, M.L. (1995). An in vitro method for determination of tissue partition coefficients of non-volatile chemicals such as 2,3,7,8-tetrachlorodibenzo-p-dioxin and estradiol. *J. Appl. Toxicol.* **15**, 147-152.

Owen, P.E., et Glaister, J.R. (1990). Inhalation toxicity and carcinogenicity of 1,3-butadiene in Sprague-Dawley rats. *Environ. Health Persp.* **86**, 19-25.

Owen, P.E., Glaister, J.R., Gaunt, I.F., et Pullinger, D.H. (1987). Inhalation toxicity studies with 1,3-butadiene. 3. Two year toxicity/carcinogenicity study in rats. *Am. Ind. Hyg. Ass. J.* **48**, 407-413.

Pang, K.S., Rowland, M., et Ozer, T.N. (1978). In vivo evaluation of Michealis-Menten constants of hepatic drug-eliminating system. *Drug Metab. Dispos.* **6**, 197-200.

Paterson, S. et Mackay, D. (1989). Correlation of tissue, blood, and air partition coefficients of volatile organic chemicals. *Br. J. Ind. Med.* **46**, 321-328.

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

Poulin, P. et Krishnan, K. (1995b). An algorithm for predicting tissue:blood partition coefficients of organic chemicals from *n*-octanol:water partition coefficient data. *J. Toxicol. Environ. Health* **46**, 101-113.

Poulin, P. et Krishnan, K.(1996a). 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.

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

Poulin, P. et 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. et Krishnan, K. (1998). A quantitative structure-toxicokinetic relationship model for highly metabolised chemicals. *ATLA.* **26**, 45-59.

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

Reitz, R.H., McCroskey, P.S., Park, C.N., Andersen, M.E., et Gargas, M.L. (1990). Development of a physiologically-based pharmacokinetic model for risk assessment with 1,4-dioxane. *Toxicol. Appl. Pharmacol.* **105**, 37-54.

Ross, R., Leger, L., Guardo, R., de Guise, J., et Pike, B.G. (1991). Adipose tissue volumes measured by magnetic resonance imaging and computerized tomography in rats. *J. Appl. Physiol.* **70**, 2164-2172.

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

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

Travis, C.C. et Bowers, J.C. (1989). Protein binding of benzene under ambient exposure conditions. *Toxicol. Ind. Health* **5**, 1017-1024.

Travis, C.C. et Hattemer-Frey, H.A. (1991). Physiological pharmacokinetic models. Dans: *Statistics in Toxicology* (D. Krewski et C. Franklin, Eds.), p. 170. Gordon and Breach, New York.

Waller, CL., Evans, MV., et McKinney, JD. (1996). Modeling the cytochrome P450-mediated metabolism of chlorinated volatile organic compounds. *Drug Metab. Dispos.* **24**, 203-210.

Wickramaratne, G.A., Tinston, D.J., Kinsey, D.L., et Doe, J.E. (1988). Assessment of the reproductive toxicology of bromochlorodifluoromethane (BCF, halon 1211) in the rat. *Br. J. Ind. Med.* **45**, 755-760.

Williams, DP. et Tung, MA. (1976). Food dispersion. Dans: *Principles of food Sciences, part I, food chemistry.* (O.R. Fennema, Ed.), pp. 530-575. Marcel Dekker, New York.

Yamaguchi, T., Yabuki, M., Saito, S., Watanabe, T., Nishimura, H., Isobe, N., Shono, F., et Matsuo, M. (1996). Research to develop a predicting system of mammalian subacute toxicity (3) construction of a predictive toxicokinetic model. *Chesmosphere* **33**, 2441-2468.