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Évaluation et modélisation de l'impact de la coexposition de composés organiques volatils sur l'excrétion de leurs biomarqueurs urinaires

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Mémoire présenté à la Faculté des Études Supérieures
en vue de l'obtention du grade de Maitrise ès Sciences (M.Sc)
en Santé Environnementale et Santé au Travail

Aout 2014

Résumé

L'évaluation de l'exposition aux composés organiques volatils (COV) recourt couramment à l'analyse des métabolites urinaires en assumant qu'aucune interaction ne survient entre les composés. Or, des études antérieures ont démontré qu'une inhibition de type compétitive survient entre le toluène (TOL), l'éthylbenzène (EBZ) et le m-xylène (XYL). Le chloroforme, qui est également un solvant métabolisé par le CYP2E1, se retrouve souvent en présence des autres COV dans les échantillons de biosurveillance. La présente étude visait donc à évaluer si le chloroforme (CHL) peut lui aussi interagir avec ces COV et évaluer ces interactions au niveau de l'excrétion des biomarqueurs urinaires associés, soit l'o-crésol, l'acide mandélique et l'acide m-méthylhippurique pour TOL, EBZ et XYL respectivement. Afin d'obtenir des données humaines, cinq volontaires ont été exposés par inhalation à différentes combinaisons de COV (seuls et mélanges binaires ou quaternaires) où la concentration de chacun des composés était égale à 1/4 ou 1/8 de la valeur limite d'exposition (VLE) pour une durée de 6h. Des échantillons d'air exhalé, de sang et d'urine ont été récoltés. Ces données ont ensuite été comparées aux modèles pharmacocinétiques à base physiologique (PCBP) existants afin de les ajuster pour l'excrétion urinaire. Certaines différences ont été observées entre les expositions aux solvants seuls et les coexpositions, mais celles-ci semblent majoritairement attribuables aux remplacements de participants à travers les différentes expositions. Les valeurs de Vmax pour EBZ et CHL ont été optimisées afin de mieux prédire les niveaux sanguins de ces COV. À l'exception du modèle pour EBZ, tous les paramètres pour l'excrétion urinaire ont été obtenus à partir de la littérature. Les modèles adaptés dans cette étude ont permis de simuler adéquatement les données expérimentales.

Mots-clés : Modélisation PCBP, biomarqueurs urinaires, toluène, éthylbenzène, m-xylène, chloroforme, inhalation.

Abstract

Evaluation of volatile organic compounds (VOC) exposure commonly resorts to urinary metabolite analyses, assuming that no interaction occur between coexposed chemicals. However, previous studies have reported competitive inhibition between toluene (TOL), ethylbenzene (EBZ) and m-xylene (XYL). Chloroform, which is also metabolized by CYP2E1, is also often found in human biomonitoring samples along with the mentioned VOCs. The goal of the present study was to evaluate if chloroform (CHL) can interact with previous VOC and to evaluate those interactions at the urinary biomarker excretion level for corresponding metabolites, namely o-cresol, mandelic acid and m-methylhippuric acid for TOL, EBZ and XYL respectively. To obtain human data, five male volunteers were exposed by inhalation to different VOC combinations (single and binary or quaternary mixtures) where concentration of each chemical was equal to 1/4 or 1/8 of the threshold limit value (TLV) for 6h. Exhaled air blood and urine samples were collected. These data were then compared with existing physiologically based pharmacokinetic (PBPK) model predictions for adjustment for urinary excretion. Some differences were observed between single and mixed exposures but they may be mainly related to volunteer replacements throughout experiments. Vmax values for EBZ and CHL were optimized to better fit blood data. Except for EBZ model, all urinary excretion parameters were taken from the literature. Models adapted in the present study adequately simulated experimental data.

Keywords : PBPK modeling, urinary biomarkers, toluene, ethylbenzene, m-xylene, chloroform, inhalation.

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Liste des sigles et abréviations

ACSLX : *Advance computer science language eXtreme*

ATSDR : *Agency for Toxic Substances and Disease Registry*

BW: Poids corporel

Ca : Concentration artérielle

CHL : Chloroforme

COV/VOC : Composé organique volatil

Cv : Concentration veineuse

CYP : Cytochrome P450

CYP1A2 : Cytochrome P450 1A2

CYP2B6 : Cytochrome P450 2B6

CYP2E1 : Cytochrome P450 2E1

EBZ : Éthylbenzène

ECD : *Electron capture detector*

FID : *Flame ionization detector*

GC : Chromatographe à phase gazeuse

Km : Constante de Michaelis-Menten

MA : Acide mandélique

MHA : acide m-méthylhippurique

MS/MS : Spectrométrie de masse en tandem

O-CR : o-Cresol

PBPK : *Physiologically based pharmacokinetic*

Prb : Coefficient de partage air:sang

Qc : Débit cardiaque

Qalv : Dévit alvéolaire

Qf, Qg, Qr, Qmp : Débit vers chacun des compartiments du modèle

SPME : Micro-extraction en phase solide

TCPB : Toxicocinétique à base physiologique

TOL : Toluène

Vmax : Vitesse maximal du métabolisme

Vt : Volume du tissu

XYL : m-Xylène

Remerciements

J'aimerais remercier mon directeur Sami Haddad, dans un premier temps pour m'avoir accordé sa confiance en me confiant ce projet. Je le remercie aussi pour son temps, ces encouragements et ses conseils. Ce n'est qu'un début, on se revoit au doctorat la semaine prochaine!

Ensuite, je remercie tous les membres du département, tout spécialement ceux "d'en haut", Igor, Mylène, Marjorie, Josée, Stéphanie, Cyril, Jonathan et Roberto pour leur soutient, pour les sorties, le plaisir, l'humour parfois douteux (Cyril) et les souvenirs.

Je remercie aussi les gens qui m'ont encadrée académiquement, Michèle et Robert. Et surtout, je remercie Ginette pour le transfert de son savoir, sans quoi on y serait encore! Je remercie également tous mes participants, à l'exception de ceux qui ne sont jamais venu, et particulièrement Rémi pour l'avoir fait à contrecœur pour répondre à mon désespoir! Merci aussi à Annabelle pour avoir pris soin d'eux.

Bien sûr, je remercie Santé Canada pour le financement et plus particulièrement Andy et Rocio pour leur collaboration. Merci également à l'IRSST pour l'octroi d'une bourse d'études et le RRSE pour l'octroi d'une bourse de présentation.

Merci à Barry et Novak, mes grosses boules de poils adorées qui me permettent de me changer les idées en dehors du bureau et Fred pour son soutien psychologique. Je crois que je me dois, avant de terminer, de me remercier, pour avoir changé d'idée à la dernière minute et foncé dans ce projet. J'avais dit que jamais je ne ferais une maitrise et voilà! J'ai dit la même chose pour le doctorat et voilà encore...doctorat en septembre!

Chapitre 1. Introduction générale

1.1 Composés organiques volatils (CTEX)

1.1.1 Définition

Les composés organiques volatiles (COV) correspondent à des molécules composées d'au moins un atome de carbone et d'un atome d'hydrogène. L'arrêté du 2 février 1998 définit les COV comme «tout composé organique, à l'exception du méthane, ayant une pression de vapeur supérieure ou égale à 0,01 kPa à une température de 293,15 K (20^{o}C) ou ayant une volatilité correspondantes dans des conditions d'utilisation particulières (pression et de température)» (Ineris). Les COV d'intérêt dans cette recherche sont le toluène, l'éthylbenzène, le m-xylène et le chloroforme. Les trois premières substances possèdent un noyau benzoïque et font partie des hydrocarbures aromatiques.

1.1.2 Utilisation et exposition

Les hydrocarbures aromatiques présentés précédemment présentent un usage semblable. On les retrouve dans les carburants et autres dérivés du pétrole, dans les peintures, les colles, l'encre et de nombreux produits d'entretien. On les emploie aussi largement comme solvants et pour la production d'autres produits chimiques (IARC, 1989; ATSDR, 1997). Le toluène est employé dans la synthèse du benzène, l'éthylbenzène dans celle du styrène (99% des volumes produits) et le m-xylène dans la synthèse d'acide phtalique ensuite utilisé pour produire des résines de polyester (Fishbein, 1988). Quant au chloroforme, la majorité des volumes produits, soit environ 95%, est utilisée dans la synthèse du Freon® (EPA, 2009). Il est aussi employé comme solvant.

Comme leur nom l'indique, les COV sont des substances qui se volatilisent généralement assez facilement à température ambiante. Le toluène, l'éthylbenzène et le m-xylène peuvent donc se retrouver dans l'air ambiant en se volatilisant à partir de produits domestiques de nettoyage ou de quincaillerie qui en contiennent. Ce type d'utilisation

entraine les plus hautes concentrations d'exposition dans les maisons. Les peintres ou les personnes travaillant en imprimerie sont généralement plus exposés. Les travailleurs œuvrant en industrie pour la production ou l'utilisation de ces solvants sont aussi plus à risque. Également, parce qu'on les retrouve dans les carburants, ces substances peuvent être relâchées dans l'air via la combustion automobile ou autre. C'est d'ailleurs la principale source d'exposition pour la population en général. La fumée de cigarette est aussi une source majeure d'exposition aux COV. Une étude réalisée par Santé Canada et l'Université de Windsor en 2005-2006 dans le cadre de la Stratégie pour la Qualité de l'Air Transfrontalier a permis d'effectuer différentes mesures, dont certaines permettant de comparer les niveaux d'exposition pour l'air intérieur, l'air extérieur et l'air personnel (Health Canada, 2010a). Selon ces données, les concentrations intérieures pour le toluène, l'éthylbenzène et le m-xylène sont en moyenne 4 à 5 fois plus élevées que les concentrations extérieures. Des tendances similaires ont aussi été observées pour la ville de Régina (Health Canada, 2010b). La source d'exposition majeure au chloroforme ne vient pas de ses principaux usages, mais du fait que cette substance représente un des principaux dérivés formés suite à la chloration de l'eau. Les piscines et l'eau de consommation représentent donc des sources importantes d'exposition au chloroforme. Bien entendu, ces concentrations environnementales sont inférieures à 1 ppm (Rappaport et Kupper, 2004). Certaines activités comme la cigarette, mettre de l'essence dans un véhicule ou l'utilisation de produits contenants de fortes concentrations de solvants (produits nettoyants, crayons marqueurs, etc) peuvent entrainer une exposition au-delà de 1 ppm pour quelques minutes (Backer *et al.*, 1997 ; Lim *et al.*, 2014). Dans les milieux de travail plus à risque comme les industries pétrochimiques, les imprimeries ou une usine de production d'adhésifs, les concentrations moyennes sont généralement inférieures à 1 ppm, bien que les concentrations maximales puissent parfois approcher les valeurs limites d'exposition permises (Kawai *et al.*, 2008).

1.1.3 Toxicocinétique

1.1.3.1 Absorption

Lors d'expositions par inhalation à des niveaux faibles de toluène (200-300 mg/m3), 50% de la dose inhalée est retenue (Löf *et al*, 1993; Carlsson, 1982). Une exposition à 188 mg/m^3 pour 90 minutes a résulté en la rétention de 86% de la dose inhalée (Benoit *et al*, 1985). Le toluène peut également être absorbé via la voie orale ou à travers la peau (Baelum *et al*, 1993; *Brown et al*, 1984; Aitio *et al,* 1984) comme pour l'éthylbenzène (El Masry *et al*, 1956; Dutkiewicz et Tyras, 1967; Gromiec and Piotrowski, 1984) et le m-xylène (Ogata *et al*, 1979; Loizou *et al*, 1999). L'exposition par inhalation à 23-85 ppm d'éthylbenzène a donné lieu à la rétention de 64% de la dose inhalée (Bardodejet and Bardodejova, 1970) et une rétention de 55% a été rapportée pour une exposition par inhalation à 4-46 ppm (Gromiec et Piotrowski, 1984) La variabilité interindividuelle ainsi que les différences méthodologiques peuvent avoir contribué à ces différences dans la rétention des composés inhalés. À des concentrations modérées (45-220 ppm), la rétention du xylène représente 59-65% de la dose inhalée (Astrand *et al*, 1978; Bardodej, 1964; Sedivec et Flek, 1976; Riihimaki *et al*, 1979a; Riihimaki *et al*, 1979b). La quantité de chloroforme absorbée à la suite lors d'exposition par inhalation n'est pas documentée (ATSDR, 1997).

1.1.3.2 Distribution

Dans le sang, le toluène se répartit entre le plasma et les globules rouges dans un ratio approximatif de 40:60 (IARC, 1989). Dans le cas d'expositions par inhalation, l'accumulation de toluène au cerveau semble être plus importante qu'au foie (ATSDR, 2000). La distribution de l'éthylbenzène et du m-xylène chez l'humain est encore peu documentée, mais ceux-ci se retrouvent dans une grande majorité de tissus. Lors de coexpositions à ces deux solvants, le ratio dans l'air inhalé est le même que celui retrouvé dans le tissu adipeux (Engstrom *et al*, 1984; Engstrom et Bjurstrom, 1978). Pour le xylène, le tissu adipeux

présente la plus grande affinité et représente 10-20% de la dose (Riihimäki et Savolainen, 1980). Le chloroforme inhalé se distribue en ordre d'importance dans le tissue adipeux, le cerveau, le foie, les reins, le sang (ATSDR, 1997). Ces COV peuvent tous traverser la barrière placentaire.

1.1.3.3 Métabolisme

L'hydroxylation du groupement méthyl du toluène est la première étape menant à la formation d'acide hippurique qui représente environ 80% de la dose absorbée (Ogata, 1984; Löf *et al*, 1993; Tardif *et al*, 1998). Cette étape est catalysée majoritairement par le CYP2E1, mais également par d'autres enzymes, notamment les CYPs 2B6, 2C8, 1A2, 1A1 (Tassaneeyakul *et al*, 1996; Nakajima *et al,* 1997; Kim *et al*, 1997). Les deux autres métabolites existants sont l'o-crésol et le p-crésol, tous deux provenant de l'hydroxylation aromatique du toluène et représentant moins de 5% des métabolites formés. Le p-crésol serait formé par les CYP2E1, 2B6 et 1A2, alors que l'o-crésol serait formé exclusivement par le CYP1A2 (Nakajima *et al*, 1997). Entre 7% et 20% du toluène absorbé est excrété inchangé dans l'air expiré (Carlsson, 1982; Lof *et al*, 1993).

L'hydroxylation du groupement éthyl de l'éthylbenzène se fait via l'action des CYP2E1 (principalement) et 2B6 (Sams *et al*, 2004) pour former le 1-phényléthanol. Celui-ci est ensuite oxydé dans des étapes plus ou moins connues (Figure 2) pour former l'acide mandélique (64-71%) et l'acide phénylglyoxylique (19-25%) (Engström *et al*, 1984; Tardif *et al*, 1997; Bardodej et BArdodejova, 1970). Engström *et al* (1984) rapportait une valeur d'environ 90% de la dose métabolisée pour ces deux métabolites (Figure 1.2). Ici encore, l'hydroxylation aromatique de l'éthylbenzène est une voie mineure du métabolisme, soit environ 4% des métabolites (Engstrom *et al*, 1984).

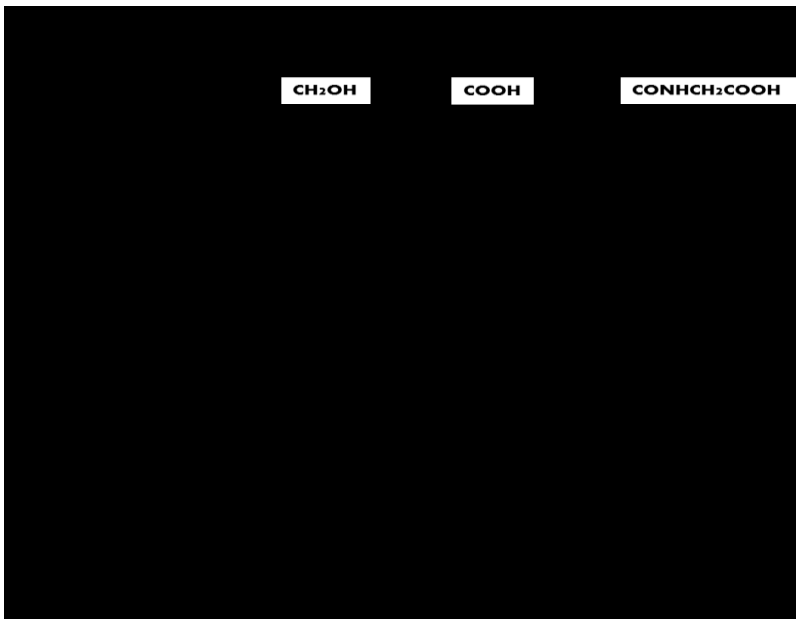

Figure 1.1 Voies métaboliques du toluène

Environ 90% de la dose absorbée de m-xylène est hydroxylé au niveau du groupement méthyl (CYP2E1) pour former ultérieurement l'acide m-méthylhippurique qui représente 95-97% de la dose métabolisée (Riihimaki et al, 1979; Engstrom et al, 1984). L'hydroxylation aromatique du m-xylène donne lieu à la formation du 2,4-diméthylphénol qui équivaut environ à 2% de la dose inhalée (Riihimaki *et al*, 1979; Sedivec et Flek, 1976; Engström *et al*, 1984). Ce métabolite serait, comme l'o-crésol, formé uniquement par le CYP1A2 (Tassaneeyakul *et al*, 1996). Dans ce même ordre d'idées, une étude sur la coexposition entre le m-xylène et l'éthanol a permis d'observer une diminution de l'excrétion de l'acide m-méthylhippurique, mais pas du 2,4-diméthylphénol (Riihimäki *et al*, 1982), suggérant une voie métabolique différente. Toutefois une étude de dynamique de substrat pour le CYP2E1 (Harrelson *et al*, 2007) indique que celui-ci peut former du diméthylphénol et ce à un pourcentage comparable aux valeurs trouvées dans l'urine à la suite d'exposition par inhalation au m-xylène (3% de 2,4-diméthylphénol et 97% de méthylbenzyl alcool).

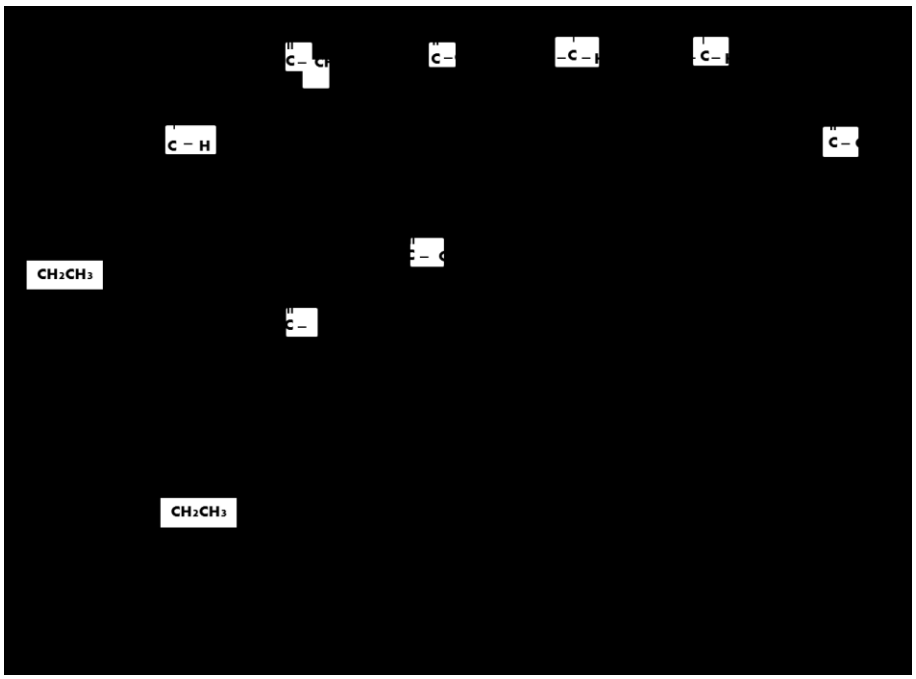

Figure 1.2 Voies métaboliques de l'éthylbenzène (Engtröm et al, 1984)

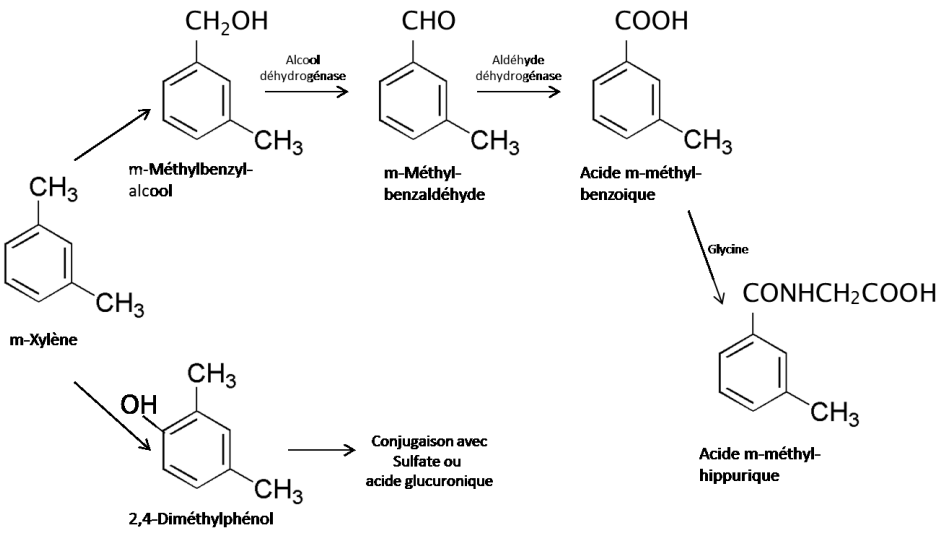

Figure 1.3 Voies métaboliques du m-xylène (Riihimäki et Haininen, 1987)

L'enzyme principale responsable du métabolisme du chloroforme est également le CYP2E1 (Wang *et al,* 1994). Cette première réaction donne naissance à un métabolite réactif qui cède alors un groupement acide hydrochlorique pour former le phosgène. De manière générale, ce dernier se lie à une molécule d'eau pour former du dioxyde de carbone et de l'acide chlorhydrique. Cependant, il peut également se lier à des macromolécules cellulaires et donc entrainer certains malfonctionnements (ATSDR, 1997). Le métabolisme du chloroforme se fait principalement au niveau du foie, mais également au niveau des reins de façon beaucoup plus négligeable.

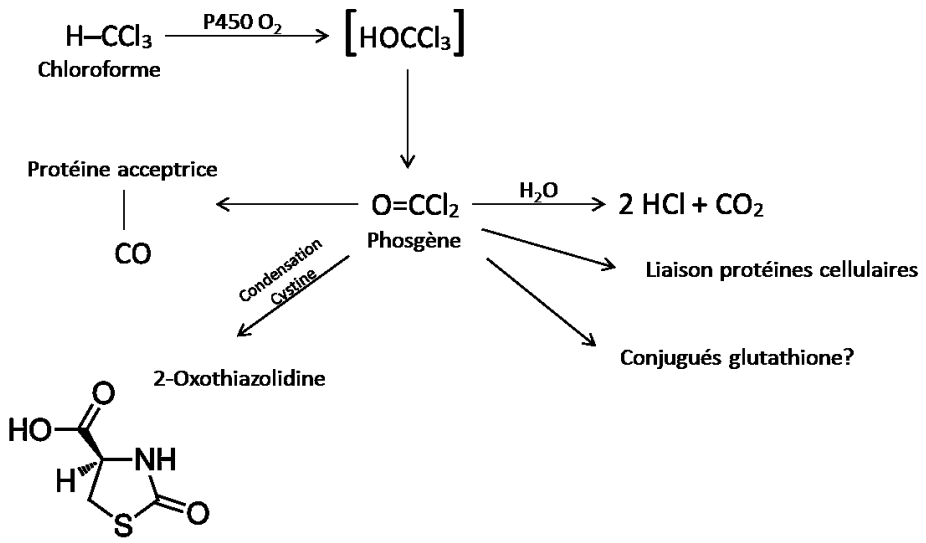

Figure 1.4 Voie métabolique (aérobie) du métabolisme du chloroforme.

1.1.3.4 Excrétion

La grande majorité du toluène est éliminée dans l'urine, sous forme d'acide hippurique. Comme mentionné précédemment, un certain pourcentage (7-20%) est éliminé inchangé dans l'air. Dans le sang, l'élimination se fait en trois phases présentant des demi-vies de 3, 40 et 738 minutes (Lof *et al,* 1993), la plus longue pouvant être attribuable à l'affinité du toluène pour le tissu adipeux.

Près de 90% de l'éthylbenzène absorbé est excrété dans l'urine sous forme d'acide mandélique et d'acide phénylglyoxylique .

Environ 4-5% du m-xylène absorbé est exhalé inchangé (Riihimaki *et al,* 1979; Sedivek et Flek, 1976; Astrand *et al*, 1978). Son excrétion comporte deux phases, une première de 3,6h et une deuxième de 30,1h (Engström *et al*, 1978). La grande majorité du m-xylène inhalé est excrété sous forme d'acide m-méthylhippurique dans l'urine.

Le chloroforme est majoritairement excrété sous forme de dioxyde de carbone ou inchangé dans l'air expiré. Une très faible quantité de chloroforme se retrouve dans l'urine (ATSDR, 1997).

1.1.4 Toxicité

Plusieurs COV comme le toluène, l'éthylbenzène, le m-xylène ou le chloroforme sont toxiques. Une exposition certaine à ces composés se traduit par des symptômes semblables pour chacun puisqu'ils ont tous pour cible principale le système nerveux central. Une exposition aigüe à l'une ou plusieurs de ces substances peut entrainer les symptômes suivant : maux de tête, nausée, irritation des yeux, du nez et de la gorge, étourdissements, vertiges, vomissements.

De manière plus précise, ces symptômes peuvent s'expliquer par une intercalation rapide de ces substances dans la bicouche lipidique des membranes nerveuses. Ce phénomène pourrait ensuite mener à des modifications biochimiques des protéines liées membranaires et ainsi altérer la transmission synaptique (Health Canada, 2014). Korpela et Tahti (1988) ont rapporté des diminutions au niveau des acétylcholinestérases, de l'ATP total et de l'activité Mg2+-ATPase dans les érythrocytes et les synaptosomes de rats exposés à de très fortes doses (2000 ppm) de toluène pour 2h. Une analyse de l'expression de gènes dans plusieurs parties du cerveau (Hester *et al,* 2011) suite à une exposition par inhalation au toluène a montré des voies associées à la plasticité synaptique altérée. Vaalavirta et Tahti (1995) ont également observé une diminution des activités Na+/K+-ATPase et Mg2+-ATPase dans des cultures d'astrocytes à la suite d'expositions à l'éthylbenzène et au m-xylène.

Des problèmes au niveau du cerveau pourraient également être attribués à des altérations au niveau des enzymes régulant les neurotransmetteurs. Il a été observé par exemple que l'exposition par inhalation au toluène peut mener à une augmentation des neurotransmetteurs (Rea *et al*, 1984 ; Aikawa *et al,* 1997) ou de la liaison à des récepteurs (Bjornaes et Naalsund, 1988). Dans le cas d'abus chroniques de toluène, des dommages irréversibles comme l'atrophie du système nerveux central ou la démyélinisation sont possibles (Borne *et al,* 2005). Les effets neurologiques tels que la perte d'audition, la dépression du système nerveux ou la narcose seraient déclenchés par le toluène lui-même et non par un de ses métabolites (ATSDR, 2000). Si la cible principale du chloroforme reste le système nerveux central, l'inhalation de ce solvant peut aussi avoir des effets néfastes sur les autres organes dont le foie et les reins (ATSDR, 1997). Ces effets peuvent être attribuables au phosgène qui se lie entre autres avec plusieurs macromolécules cellulaires au niveau de ces organes.

Il est difficile de connaitre les effets de ces substances chez l'humain à long terme étant donné que l'exposition est généralement caractérisée par la présence de solvants multiples et il devient moins évident d'attribuer des effets à l'un ou l'autre. Une exposition chronique au toluène au-dessus des niveaux permis pourrait endommager l'audition et possiblement la vision en couleur (Lomax *et al*, 2004). Des études chez le rat ont montré que l'exposition prénatale au toluène pouvait affecter la croissance des fœtus, le développement squelettique et le comportement de la progéniture (ATSDR, 2000). Des résultats semblables ont été rapportés pour l'éthylbenzène. Toujours chez le rat, une exposition chronique par inhalation à l'éthylbenzène (0, 75, 250 ou 750 ppm, 6h par jour pour une durée de 104 semaines) a entrainé une augmentation de l'incidence d'hyperplasie du tubule rénal chez les mâles et une augmentation du degré de néphropathie spontanée et progressive liée à l'âge chez les deux sexes. Chez la souris (mâle), une augmentation de l'incidence de métaplasie de l'épithélium alvéolaire, des altérations syncytiales des hépatocytes, de l'hypertrophie hépatocellulaire, de la nécrose des hépatocytes et l'hyperplasie des cellules des follicules thyroïdiens ont été observées. Chez la femelle, on rapporte une augmentation de l'incidence des foyers éosinophiles du foie, de l'hyperplasie de la glande pituitaire et des cellules des

follicules thyroïdiens (National Toxicology Program, 1999). Les données expérimentales animales soutiennent le potentiel carcinogène de l'éthylbenzène alors que les données chez l'humain sont inadéquates, ce qui classe le solvant dans le groupe 2B, c'est-à-dire comme possiblement carcinogène pour l'humain selon l'IARC. Le toluène et le m-xylène se trouve dans le groupe 3 (non classifiable quant à leur carcinogénicité chez l'humain).Pour ce qui est du chloroforme, l'EPA (1999) le classe dans la catégorie 2B également.

Les symptômes et autres atteintes mentionnés précédemment sont le résultat d'expositions relativement élevées ayant peu de chance de survenir en dehors d'un contexte expérimental. La toxicité des COV à des concentrations environnementales pour la population est mal connue. Cette lacune constitue un des éléments qui souligne la nécessité de développer des outils permettant de mieux caractériser le risque lié à ces faibles expositions.

1.1.5 Valeurs limites

Les valeurs limites d'exposition pondérées (VLEP) pour 8h d'exposition par jour pour un total de 40h par semaine permises par l'*American Conference of gouvernemental industrial hygienists* (ACGIH) en 1995 et 1996 et employées dans la présente étude sont de 10, 50, 100 et 100 ppm pour le chloroforme, le toluène, l'éthylbenzène et le m-xylène respectivement. Certaines de ces valeurs ont été réajustées en fonction des résultats observés dans certaines études mentionnées ci-haut, notamment les études neurologiques (ACGIH, 2006). En 2007, la valeur limite pour le toluène est passée à 20 ppm tout comme celle pour l'éthylbenzène en 2011. Pour des fins de comparaison avec d'autres données expérimentales, les valeurs antérieures ont été retenues.

1.2 Biosurveillance des COV

1.2.1 En quoi consiste la biosurveillance?

Prévoir les concentrations d'une substance chimique dans l'organisme en se basant sur des valeurs mesurées dans l'air, l'eau, le sol, les aliments ou d'autres produits de consommation n'est pas simple, car cela nécessite d'estimer les habitudes de chacune des personnes exposées, leur mode de vie et la cinétique des substances chimiques auxquelles elles sont exposées. La biosurveillance consiste à évaluer les concentrations internes de ces substances et de leurs dérivés à l'aide d'analyses dans le sang, l'urine, les cheveux, la salive, le lait maternel, etc. Ces méthodes ne sont généralement pas ou peu invasives et permettent d'éviter les erreurs d'estimation. Cette pratique permet entre autres de s'assurer que les expositions ne dépassent pas les valeurs limites d'exposition permises en comparant les valeurs obtenues dans les matrices biologiques avec les valeurs maximales attendues pour une exposition maximale permise.

1.2.2 Méthodes actuelles

Les méthodes habituelles de biosurveillance se basent régulièrement sur les corrélations existantes entre la concentration d'une substance dans le corps et les concentrations d'exposition (régression linéaire). Dans le cas du toluène par exemple, il a été observé que la concentration en o-crésol, un métabolite du toluène, dans l'urine était hautement corrélée à la concentration de toluène dans le sang (R=0,99) et dans l'air (Apostoli *et al,* 1982). Un autre métabolite, l'acide hippurique est lui aussi souvent rapporté comme ayant une forte corrélation avec le toluène dans le sang et dans l'air. Cependant, l'acide hippurique peut être produit de façon endogène et plusieurs facteurs comme l'alimentation peuvent en modifier les niveaux (Tardif *et al*, 1998). Ainsi, il a été observé que l'o-crésol

urinaire est davantage corrélé à l'exposition que l'acide hippurique pour des concentrations d'exposition inférieures à 50 ppm, soit la valeur limite d'exposition.

En ce qui a trait à l'éthylbenzène, c'est habituellement son métabolite principal, l'acide mandélique, qui sert à l'évaluation de l'exposition. L'acide mandélique, en plus d'être le métabolite majeur suite au métabolisme de l'éthylbenzène, n'est pas présent de façon endogène dans le corps. Par contre, l'acide mandélique est également un métabolite du styrène, ce qui peut compliquer l'interprétation des niveaux d'exposition lorsque le styrène et l'éthylbenzène se retrouve tous deux dans l'environnement évalué.

Pour le m-xylène, l'acide m-méthylhippurique s'avère un très bon bioindicateur puisqu'il représente la majeure partie des métabolites, soit près de 97% (Engström *et al,* 1984) et qu'il n'est pas présent dans le corps humain en absence d'exposition au xylène.

1.2.3 Limites

Parce qu'elle se base principalement sur des corrélations, la biosurveillance présente certaines limites. Il a été démontré principalement au cours des 30 dernières années que la coexpostion à des composés comme les COV, pouvait entrainer des variations au niveau sanguin et dans l'air exhalé des concentrations en substances mères, de même que des changements dans l'excrétion des métabolites (Tardif *et al*., 1997; Haddad *et al*., 1999). Ces changements viennent alors fausser les estimations issues des corrélations sur lesquelles se base la biosurveillance de ces substances chimiques. Il existe une autre façon de relier les valeurs biologiques aux expositions tout en tenant compte de la capacité potentielle d'une substance à modifier la cinétique d'une autre substance et cette solution est appelée «modélisation toxicocinétique à base physiologique».

1.3 Modélisation TCBP

1.3.1 Concept de la modélisation

La modélisation toxicologique (ou pharmacologique) à base physiologique est une approche visant à représenter le corps humain (ou animal) par différents compartiments (organe, tissus, etc.), lesquels sont reliés par des équations différentielles décrivant la cinétique d'un composés à travers ces différents compartiments. Ce type de modélisation a la particularité d'intégrer plusieurs variables biologiques et physiologiques comme des coefficients de partage tissu:sang, le débit de ventilation ou cardiaque, le poids, le métabolisme, etc. Si l'usage de ces modèles étaient autrefois limité au développement de médicaments dû à leur complexité mathématique et au grand nombre de données *in vivo* provenant de tissus animaux nécessaires, nos capacité actuelles à prédire les capacités métaboliques et la distribution aux tissus à partir de données *in vitro* et *in silico* rendent cet usage beaucoup plus aisé et intéressant (Jones et al, 2009). En plus de permettre l'estimation de paramètres cinétiques et les concentrations tissulaires d'un composé chimique, les modèles TCBP peuvent également permettre d'évaluer la variabilité dans la susceptibilité d'une population (Blois *et al*, 2014; Krauss *et al*, 2013).

Lors d'une exposition à une substance chimique par inhalation, les poumons représentent l'entrée de la substance, son absorption étant régie par le coefficient de partage sang:air de celle-ci, par le débit de ventilation et par le débit cardiaque. L'absorption d'une substance par un tissu peut être limitée par la perfusion (le débit cardiaque), comme c'est souvent le cas pour les molécules ayant un faible coefficient de partage tissu:sang ou être limitée par la ventilation alvéolaire, qui est plutôt le cas des substances ayant moins d'affinité pour le sang (NLM). Les concentrations artérielle et veineuse sont représentées par les équations suivantes :

$C_A = (Q_c * C_V + Q_{alv} * C_{inh}) / (Q_c + (Q_{alv} / P_{rb}))$

$C_V = (Q_f \times C_{Vf} + Q_g \times C_{Vg} + Q_r \times C_{vr} + Q_{mp} \times C_{Vmp}) / Q_c$

où C_A et C_V représentent la concentration artérielle et la concentration veineuse respectivement, les débits cardiaque et alvéolaire étant définis par Q_c et Q_{alv}. C_{inh} est la concentration inhalée et P_{rb} le coefficient de partage sang :air de la substance. Q_f, Q_g, Q_r et Q_{mp} représentent le débit au foie, aux tissus adipeux, aux tissus richement perfusés et aux tissus moyennement perfusés respectivement. C_{Vf}, C_{Vg}, C_{Vr} et C_{Vmp} représente la concentration veineuse pour chacun de ces compartiments. Les concentrations dans ces compartiments peuvent être décrites selon deux équations, selon qu'il y ait métabolisme (biotransformation de la substance chimique inhalée) ou non.

Sans métabolisme : $\frac{dCt}{dt} = (Q_t \times C_A - Q_t \times C_{Vt}) / V_t$

Avec métabolisme : $\frac{dCt}{dt} = (Q_t \times C_A - Q_t \times C_{Vt} - ((V_{max} \times C_{Vt}) / (K_m + C_{Vt}))) / V_t$

où C_t est la concentration dans le tissu, V_t est le volume du tissu, Q_t et C_{Vt} sont le débit et la concentration veineuse du tissu et V_{max} et K_m représentent la vitesse maximal du métabolisme et la constante de Michaelis Menten respectivement. Les débits alvéolaire et cardiaque, de même que la vitesse du métabolisme dépendent du poids corporel et sont représentés selon la relation suivante :

$$V_{max} = V_{maxC} \times BW^{0.75}$$

où V_{maxC} est exprimé en mg/h/kg et BW est le poids corporel (Krishnan et Andersen, 1991).

Ces équations forment la base de la modélisation pour une exposition par inhalation. En fonction des différentes voies métaboliques pouvant être impliquées (conjugaison au GSH, métabolisme rénale, excrétion biliaire, etc.) et de la voie d'absorption, plusieurs autres paramètres et équations peuvent être ajoutés.

1.3.2 Outil pour les mélanges

Outre les avantages de la modélisation TCBP mentionnés auparavant, celle-ci permet également de jeter un regard sur les mécanismes derrière la cinétique. Ces mécanismes sont importants pour l'interprétation des effets néfastes d'une substance chimique dans l'organisme et peuvent aussi permettre de prédire le comportement de substances similaires, d'aider à établir des valeurs limites d'exposition ou encore de prédire les effets pouvant résulter d'une coexposition simultanée à plus d'un composé. Il devient maintenant de plus en plus reconnu que les études sur les mélanges binaires peuvent, lorsqu'interconnectées dans un modèle TCBP, prédire les interactions issues de mélanges de plusieurs substances (Tardif *et al,* 1997; Haddad *et al*, 1999, 2000).

1.4 Problématique

Cette étude s'inscrit dans le cadre l'Enquête Canadienne sur les Mesures de Santé (ECMS). Cette étude de grande envergure vise à obtenir de l'information notamment sur l'exposition de la population aux COV. Cependant, afin de corréler les valeurs mesurées à des niveaux d'exposition, il est nécessaire de développer des outils permettant de tenir compte des interactions potentielles entre les différents composés d'intérêt.

1.5 Objectifs de l'étude

L'objectif principal de cette étude était donc de fournir ces outils d'évaluation en adaptant les modèles TCBP existants pour le chloroforme, le toluène, l'éthylbenzène et le m-xylène afin de prédire les concentrations sanguines et alvéolaires pour les substances mères et les niveaux urinaires des biomarqueurs associés suite à des coexpositions à ces solvants.

Pour ce faire, il était nécessaire dans un premier temps de récolter des données expérimentales auprès de volontaires à la suite d'expositions aux substances seules ou en mélange. Les données issues des expositions simples devaient ensuite servir à ajuster les modèles TCBP afin de décrire l'excrétion urinaire des biomarqueurs (article 1).

Dans un deuxième temps, les données pour les mélanges binaires avec le chloroforme devaient servir à évaluer les possibles interactions entre ce composé et les autres solvants étudiés pour ensuite être intégrées dans un modèle pour les mélanges quaternaires afin de valider les modèles ajustés/développés dans la présente étude (article 2).

Chapitre 2 : Modélisation pharmacocinétique à base physiologique des biomarqueurs d'exposition chez l'humain suite à une exposition au toluène, à l'éthylbenzène ou au m-xylène.

2. Physiologically based pharmacokinetic modeling of exposure biomarkers in human volunteers exposed to toluene, ethylbenzene or m-xylene.

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2.1 Abstract

Urinary biomarkers of exposure are used widely in biomonitoring studies. The commonly used urinary biomarkers for the aromatic solvents toluene (T), éthylbenzène (E) and m-xylene (X) are o-cresol, mandelic acid and m-methylhippuric acid. The toxicokinetics of these biomarkers following inhalation exposure have yet to be described by PBPK modeling. To accomplish that objective, five male volunteers were exposed for 6 hours in an inhalation chamber to 1/8 or 1/4 of the TLV® for each solvent: Toluene, ethylbenzene and m-xylene were quantified in blood and exhaled air and their corresponding urine biomarkers were measured in urine. Published PBPK model for parent compounds were used and simulations were compared to experimental blood and exhaled air concentration data. When discrepancies existed, Vmax and Km values were optimized to better describe the experimental data. A Vmax of 13,32 mg/h/kg was obtained for ethylbenzene. Urinary excretion was modeled using parameters found in literature assuming simply stoichiometric yields from parent compound metabolism and first order urinary excretion rate. Alternative models were also used to test i) the possibility that CYP1A2 is the only enzyme implicated in o-cresol formation, ii) the utilization of a 3 steps model for describing metabolic pathway for mandelic acid. Mandelic acid levels were lower than expected from literature while m-methylhippuric acid levels were higher than expected. Models adapted in this study for urinary excretion will be further used to interpret urinary biomarker kinetic data from mixed exposures of these solvents.

Keywords: biomarkers, PBPK modeling, inhalation, toluene, ethylbenzene, m-xylene.

2.2. Introduction

Determination of metabolites in urine is a common practice to assess exposure to volatile organic compounds. However those measurements are usually based on data for single exposure and do not consider possible interactions that may occur between co-exposed chemicals. Although former studies on toluene, ethylbenzene and m-xylene revealed no shifts in blood levels of parent compounds at low exposure levels during co-exposures (Tardif *et al,* 1991; Tardif *et al,* 1995), there is still a dearth of information regarding metabolic interaction impact on urinary metabolites.

Physiologically based pharmacokinetic (PBPK) modeling is a useful approach to study the possible mechanisms behind those interactions. Some studies have been conducted to investigate the effect of possible interactions between solvents on the levels of metabolites in urine (Engtröm *et al*, 1984; Elovaara *et al*, 1984; Tardif *et al,* 1997) but none has ever integrated those results to a PBPK model to simulate interactions at the urinary metabolites level. Previous studies investigating blood levels would only be representative of interactions occurring at the first step of metabolism if interactions occurring in subsequent steps are not to modulate blood levels of unchanged chemicals. Such blood levels are useful to evaluate the real toxicity risk when parent compounds cause the toxic effects but urinary metabolites are more useful in a biomonitoring approach because of their accessibility.

A PBPK model for o-cresol excretion has been proposed by Tardif *et al* (2002). m-Xylene metabolite excretion has been studied (Engström *et al*,1978; Riihimäki *et al*, 1987; Riihimäki, 1979) but the resulting kinetic parameters (excretion rate and fraction of metabolism for each metabolite) have not been integrated to a PBPK model. Loizou *et al* (1999) did use some of that information to model methylhippuric acid excretion. In the case of mandelic acid, no model or excretion rate could be found in literature.

Because our ultimate goal is to predict impact of co-exposures on the kinetics of urinary metabolites used as biomarkers of exposures to toluene (T), ethylbenzene (E) and m-xylene (X), this study will focus on developing PBPK models for the metabolites of toluene, ethylbenzene and m-xylene for single chemical exposures. The objective of the present study was therefore to collect human data from controlled inhalation exposures in order to refine existing PBPK models by integrating urinary excretion kinetics of their metabolites for single exposures and to validate these models. This study represents the first step in interpreting and modeling interactions occurring between common volatile organic compounds to predict their impact on their urinary level.

2.3 Material and methods

2.3.1 Volunteer exposures and chemical analyses

2.3.1.1 Material

Toluene (99.8% purity), ethylbenzene (99% purity) and m-xylene (≥99% purity) were purchased from Sigma Aldrich. o-cresol (≥99% purity), m-methylhippuric acids (98% purity), mandelic acid (99% purity), hippuric acid (98% purity), o-cresol-d^8, 2,3,4,5,6-pentafluorobenzyl bromide (PFB-Br) (99% purity), tetrabutylammonium hydrogensulphate (TBA), methanol (≥99% purity), dichloromethane (≥99% purity), sodium hydroxide (1M), sulfuric acid (0,5 M), $NaH_2PO_4.H_2O$ were purchased from Sigma Aldrich. Mandelic-2,3,4,5,6-d^5 acid was purchased from CDN Isotope Inc. (Pointe-Claire, Canada). Toluene-$^{13}C_6$, Ethylbenzene-$^{13}C_6$ and m-xylene-^{13}-C_2 were purchased from Cambridge Isotope Laboratories Inc (Andover, MA).

2.3.1.2 Volunteers

Five male volunteers aged between 18 and 35 and weighting between 58 and 100 kg participated in this study. They were non-smokers with no recent history of exposure to high levels of VOCs or health issues (assessed by a questionnaire). They were asked not to consume alcohol for a 24h period before and after exposure. They provided their informed consent and the project was approved by the Ethics Committee of the Université de Montréal and Health Canada.

2.3.1.3 Exposure chamber

The human exposure chamber system located in the Inhalation Laboratory of the Department of Environmental and Occupational Health of the Université de Montréal has previously been described by Tardif *et al.* (1991). Solvent concentrations in air were generated by introducing with an HPLC pump (Varian 2510) the solvents in a round –bottom

flask and mixing them with a stream of purified air. This air then passed through 16 diffusers in the chamber ceiling. Concentrations in the chamber air were monitored every 10 minutes by directly sampling the air from the chamber with a pump connected to a gas chromatograph. Concentrations were also continuously monitored by a Miran205B Series SapphIRe infrared spectrophotometer (CD Nova Ltd., Burnaby, British Columbia, Canada). Mean variation of concentrations in chamber during each experiment was 5%.

2.3.1.4 Exposures and analytical methods

Volunteers were exposed to ¼ and 1/8 of TLV (ACGIH, 1995-1996) of each solvent. They were exposed for 6 h/day with one-week interval between each day of exposure. TLVs are 50 ppm for toluene and 100 ppm for ethylbenzene and m-xylene.

Exhaled air samples were collected throughout the exposure time (time points: 0, 1.5, 3, 4.5, 6, 6.5 and 7h) in 3L Tedlar bags. One blood sample was collected 5 min. before exposure to measure background exposure and subsequent samples were collected after the end of exposure (15, 45, 90 and 120 minutes after the end of exposure). A catheter was installed to facilitate sampling. Samples were then kept refrigerated until analysis. Total urine was collected before, during (3.5 h) and after exposure (8, 15 and 24h after start of exposure) and then stored at -20^{o}C for further analysis.

Alveolar air, air from the chamber, blood and urine samples were analyzed according to analytical methods described in the appendix section.

2.3.2 PBPK modeling of parent compounds and metabolites

2.3.2.1 Model representation

Parent compound

PBPK model for each chemical is represented as a tissue network composed of richly perfused tissues (RPT), slowly perfused tissues (SPT), adipose tissues and liver (Figure 1).

These four compartments are interconnected by systemic circulation and gas exchange in the lung. Metabolism is limited to the liver and is described by Vmax (mg/h) and Km (mg/L). All kinetics (except for metabolites excretion) are described by Ramsey and Anderson equations (1984).

Urinary biomarkers

In Figure 2a, metabolism for each monitored metabolite is represented. o-Cresol is first represented as a fraction of the toluene metabolism by CYP2E1 (CYP2E1 model) (Tardif *et al*, 2002). Amount of o-cresol in urine is therefore described by a stoichiometric yield (SY) and an excretion constant (K_{CRE}). On the other hand, literature suggests that o-cresol is formed by CYP1A2 activity (Nakajima *et al,* 1997; Tassaneeyakul *et al,* 1996; Kim *et al*, 1997). An alternate description (Figure 2b) for o-cresol was therefore suggested (CYP1A2 model) by using a first order metabolic rate constant (K_{TOL}) for its formation by CYP1A2 and a first order rate constant for its urinary excretion (K_{CRE}).

A first model (1-step model) was proposed to describe mandelic acid excretion with a stoichiometric yield parameter and a first order excretion rate constant (K_{MA}) (Figure 2 c). Because literature mentions the existence of a rate limiting step (Engström *et al,* 1984), a second representation for mandelic acid formation and excretion (Figure 2d) is proposed (2-step model) and is composed of two different steps, the first one being described as a saturable process with Vmax and Km values and the second as a first order rate constant.. The first step is the conversion of ethylbenzene to to mandelic acid which can be converted to phenylglyoxylic acid (second step) or excreted in urine with a first order rate constant (K_{MA}) .

Finally, the model for m-methylhippuric acid (Figure 2e) is described by a stoichiometric yield component followed by a first order excretion rate constant (K_{MHA}).

2.3.2.2 Model parametization

Parent compound

Physiological parameters (Table 2) for all three compounds were the same and values were taken from Tardif *et al* (1997). Because Km and Vmax values from Tardif *et al* (1997) and those from Haddad *et al* (1999) were different for toluene and m-xylene, they were both tested with our data (Table 3).

Urinary biomarkers

For o-cresol, a stoichiometric yield of 0,0078 (fraction of metabolism that yields to the metabolite formation) and excretion rate 0,715 $h^{-1}kg^{-1}$ were taken from the PBPK model of Tardif *et al* (2002) (Table 4). A first order constant was also used to simulate o-cresol formation by CYP1A2. The stoichiometric yield of 0,97 for m-methylhippuric acid is the one reported by Engström *et al* (1984) and the excretion rate of $1,3h^{-1}$ was taken from Riihimäki (1979). Parameters for mandelic acid excretion in urine were estimated in this study except for stoichiometric yield for mandelic acid and phenylglyoxylic acid (0,9) which was taken from Engström *et al* (1984).

2.3.2.3 Model calibration and simulations

Results for single exposures were compared to predictions made with proposed and previously published PBPK models [i.e., Haddad *et al* (1999); for toluene and Tardif *et al*., 1997 for ethylbenzene and m-xylene]. Excretion parameter values described above were incorporated into the models (Table 5). The different mass balance differential equations representing the toxicokinetics of parent compounds and their metabolites were written as a program and solved with ACSLXtreme software (Aegis Technologies Inc, Huntsville AL). Optimizations were performed using ACSLX Optimum. Heteroscedasticity parameters were kept at 1 and the Nelder-Mead algorithm was used.

Parent compound

As a first step, simulations for parent compound kinetics in blood and air, using parameter values in Table 3, were compared to data obtained experimentally to determine if metabolic constants were appropriate to describe the data. In the case where predictions were off of experimental data range, optimization of these parameters was performed. Km values were not optimized. Optimization was made by fitting model to experimental data (best visual fit) for venous blood and exhaled air.

Urinary biomarkers

Data for urinary biomarkers were compared to model predictions. If parameters for excretion could not predict the experimental results in urine, they were optimized. When optimization was unable to provide good fit, alternative models with intermediate steps for metabolism were used for simulation and optimization as mentioned above (Figure 2b and d).

2.4 Results

Experimental data for single exposure were compared to simulations from existing models (Haddad *et al*, 1999; Tardif *et al*, 1997) and kinetic parameters for the urinary excretion of the metabolites were subsequently added and adjusted to experimental data.

The model of Tardif *et al*, (1997) overpredicted the experimental data for toluene concentration in blood while the Km and Vmax values from Haddad *et al*, model (1999) provided a better fit to the experimental data (Figure 3). Blood concentrations of ethylbenzene were also overestimated by the model of Tardif *et al*, (1997). Since data for ethylbenzene in blood tended to be lower than simulations and no other in vivo values for Vmax or Km were available in the literature, *Vmax* was optimized (Table 4). The new *Vmax* for ethylbenzene is around 50% higher (13.32) than the values reported in other in vivo

studies (7.3) (Tardif *et al*, 1997; Haddad *et al*, 1999). m-Xylene blood levels were well predicted by the Tardif model.

For toluene concentration in exhaled air (Figure 4), data obtained during inhalation are well predicted by Tardif's model but post-exposure data are overestimated. Replacement of Km and Vmax values for those from Haddad *et al* (1999) fitted the experimental data better. The change in Vmax (optimization) for ethylbenzene did not alter the capacity of the model to predict experimental data. In the case of m-xylene, if blood concentrations could be well predicted at both levels of exposure, only data for the exposure at 1/8 of the TLV were close to model predictions in exhaled air.

All models for o-cresol excretion could adequately predict our results (Figure 5). Simulations for o-cresol formation occurring via CYP1A2 and the simulation for CYP2E1 with an optimized stoichiometric yield provided a similar fit to experimental data. Original and optimized parameter values are given in Table 4.

Mandelic acid excretion in urine was slower than ethylbenzene elimination from blood. When using the 1-step model, simulations could not follow the curve suggested by measured levels of mandelic acid in volunteers, nor be optimized to do so. When using the 2-step model with formation of PGA from mandelic acid, an adequate optimization could be achieved to simulate the mandelic acid excretion data. Original and optimized parameter values are presented in Table 4.

In the case of MHA excretion, using the reported stoichiometric yield (0.97) and urinary excretion constant ($1.3h^{-1}$) for MHA, the model accurately predicted the observed results (Figure 5).

2.5 Discussion

This study allowed to extend the existing PBPK models for toluene, ethylbenzene and m-xylene to incorporate a description of the excretion kinetic of their urinary biomarker with results for single exposures. Predictions from existing models were first compared with experimental data for blood and alveolar air. An excretion constant and a stoichiometric yield for each biomarker were then added to validated models in order to predict urinary excretion levels of these biomakers. When metabolite excretion could not be predicted properly, alternative models were investigated.

2.5.1 Model predictions vs experimental data for parent compounds in blood and alveolar air.

The high level of exposure (1/4 TLV) in our study is relatively close to the exposure level published in Tardif *et al* (1997) (i.e., 12,5 ppm vs 17 ppm respectively for toluene and 25 ppm vs 33 ppm for ethylbenzene and m-xylene). No similar time points were used in both studies except for measurement of air concentrations. Experimental data can however be compared to predictions from Tardif *et al* (1997) model.

Tardif's model did not properly predict experimental data in this study for toluene concentration in blood but did predict adequately exhaled air concentrations during exposure, post-exposure data being overestimated. Because of these discrepancies, Vmax and Km values from Haddad *et al* (1999) were then tested. They were more accurate for toluene levels in exhaled air (post exposure data) and blood (Figures 3 and 4) compared to Tardif's model and were kept for urinary excretion of o-cresol modeling. Vmax and Km values from Haddad *et al* (1999) are based on a rat study just like those from Tardif for toluene (Tardif *et al*, 1995). The fact that Tardif *et al* (1997) had two time points during the exposure compared to Haddad *et al* (1999) and this study where all samples were collected after end

of exposure could have contributed to the difference observed in Vmax and Km values between studies.

Exhaled air result after 6h of inhalation for the exposure to ethylbenzene is equal to 72% of that from Tardif *et al* (1997) which nicely reflects the 75% difference in exposure levels in both studies. However, using Tardif values for Vmax and Km, the PBPK model overestimated venous blood concentrations for ethylbenzene (Figure 3). Therefore new values were optimized as no other published *in vivo* values with a higher intrinsic clearance existed for humans. Km was kept unchanged because optimization of both parameters simultaneously always gave the same Vmax/Km ratio regardless of their values, thus suggesting the absence of saturation at these exposure levels. A Vmax value of 13.32 mg/h/kg was obtained. Interestingly, after conversion according to Lipscomb *et al* (2004) reported *in vitro* values by Sams *et al* (2004) would give a Vmax of 8.5 mg/h/kg with a Km of 0.85 mg/L. The Vmax/Km ratio of 10 is very close to the one obtained with optimized value of 13.32 mg/h/kg for Vmax and a Km of 1.39 mg/L.

Even if the model for m-xylene accurately predicted parent compound concentrations in blood samples, it had mixed success in alveolar air (i.e., good prediction at 1/8 TLV but slight underprediction at ¼ TLV). Although subject in this study where exposed at 25 ppm of m-xylene compared to 33 ppm for Tardif, values for exhaled air concentration obtained at 6h after beginning of exposure were very similar (4.7 ppm vs 4.5 ppm in this study). A 25% difference would have been expected, and this is confirmed by the mean 22% difference between experimental data and model predictions.

2.5.2 Model predictions vs experimental data for urinary biomarkers

Modeling o-cresol excretion in urine using parameters for CYP2E1 activity (Vmax and Km values), a stoichiometric yield and published excretion constant (Tardif et al, 2002) permitted

a good fit with experimental data in urine for this study as well as for Tardif *et al* study (Table 5). However, the stoichiometric yield was optimized to achieve a better fit (Table. The other possibility suggests CYP1A2 would be responsible for the aromatic hydroxylation producing o-cresol such as demonstrated in *in vitro* studies (Nakajima *et al,* 1997; Tassaneeyakul *et al,* 1996; Kim *et al*, 1997). Following that idea and because saturation of that enzyme is unlikely to occur at levels of exposure in our study, we attempted to describe o-cresol formation by a first order constant. A value of 0.122 $h^{-1} \bullet kg^{-1}$ was obtained and provided a good correlation with our data. This alternative model could be useful in interpreting possible interactions between co-exposed chemicals.

In terms of modeling, results for mandelic acid excretion did not fit very well the predictions using only a stoichiometric yield and an excretion constant (Figure 5). One plausible explanation could be that there are some intermediate limiting steps that modulate the excretion rate. Conversion of ethylbenzene to 1-phenylethanol is supposed to occur at a similar rate than m-xylene metabolism while subsequent steps would be much slower, thus limiting metabolite excretion rates (Engström *et al*, 1984). Another important aspect is that phenylglyoxylic acid is thought to be formed by ADH enzyme from mandelic acid (Engstrom *et al,* 1984). As shown in Figure 5, the addition of that step is needed to provide a good fit of our data to the urinary excretion curves of mandelic acid

The model adapted in this study was compared with experimental data (Figure 6) from a former study (Tardif et al, 1997). If predictions for blood levels for toluene were a little low near the end of exposure, the model did provide good fit for other data. All experimental data for alveolar air concentration were well predicted by the model. Only the last data for o-cresol cumulative urinary excretion could be predicted by the model. This could be due to method precision for measuring small concentrations of metabolite in urine, since we used a more accurate method. First data points for mandelic acid excretion were relatively well predicted while the 24h amount was underpredicted at 40%. When considering the theoretical amount of ethylbenzene metabolized, that mandelic acid is supposed to

represent between 55% and 70% (Gromiec and Piotrowski, 1984; Bardodej and Bardodeva, 1970; Engstrom et al, 1984) of total metabolites and that we expect about 45% of total mandelic acid to be excreted after 24h (Gromiec and Piotrowski, 1984) then we should observe between 64 and 82 mg of mandelic acid after 24h in our study and between 103 and 131mg for Tardif study. Therefore we could assume that our results are little low and that their results are a little high. Urinary results for m-methylhippuric acid were overpredicted by the model. However, reported parameters in literature for urinary excretion of m-methylhippuric acid allowed a good fit of our data with model predictions.

2.6 Conclusion

In conclusion, models developed in this study can appropriately predict urinary biomarkers for low level exposures to toluene, ethylbenzene and m-xylene. If models adjusted for urinary metabolite excretion in this study can predict human data for single exposure they still have to be tested with data from exposure to chemical mixtures. Further efforts are currently ongoing to validate these models in the context of exposure to mixtures. They could be useful for the interpretation of large scale exposure biomonitoring studies such as the Canadian Health Measures Survey (CHMS).

2.7 Acknowlegments

This research project was supported by Health Canada. The authors would like to thank Ginette Charest-Tardif, Hayet Belmeskine Jeromy Harvie and Zhiyun Jin for their technical support, Annabelle Espurt for her assistance in blood sampling,Josée Dumas-Campagna for her assistance during sample collections and finally but not least all the volunteers for their precious help.

2.8 References

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2.9 Figure captions

Figure 1. Conceptual representation of the physiologically based toxicokinetic model for toluene (T), ethylbenzene (E) and m-xylene (X). Qp and Qc refers to alveolar ventilation rate and cardiac output respectively while Qf, Qs, Qr and Ql refer to blood flows to each compartment. Cv and Ca are for venous and arterial blood concentration and Cinh and Cexh are for inhaled and exhaled air concentration. The rate of the amount metabolized (RAM) is described by the maximal velocity of of the metabolism (Vmax) and Michaëlis affinity constant (Km).

Figure 2. Conceptual representation of (A) o-cresol formation by CYP2E1, (B) o-cresol formation by CYP1A2, (C) single step mandelic acid formation, (D) mandelic acid formation with a rate limiting step and formation of phenylglyoxylic acid from mandelic acid and (E) formation of m-methylhippuric acid. SY refers to the stoichiometric yield (% of total metabolites) and KOCR, KMA and KMHA are excretion constants for o-cresol, mandelic acid and m-methylhippuric acid respectively. KTOL is a first order constant for o-cresol formation by CYP1A2. Vmax, Km and Cvl are the maximal velocity of the metabolism, the affinity constant and venous concentration for liver respectively.

Figure 3. Comparisons of PBPK model simulations (lines) and experimental data (□) of blood concentrations of toluene, ethylbenzene and m-xylene in human volunteers exposed to ¼ or 1/8 TLV by inhalation for 6 h. Simulations were run with existing models (dotted lines) and with optimized parameters (solid lines). In the case of toluene, the dotted line is for model from Tardif et al (1997) and the solid line is for Haddad et al (1999) model.

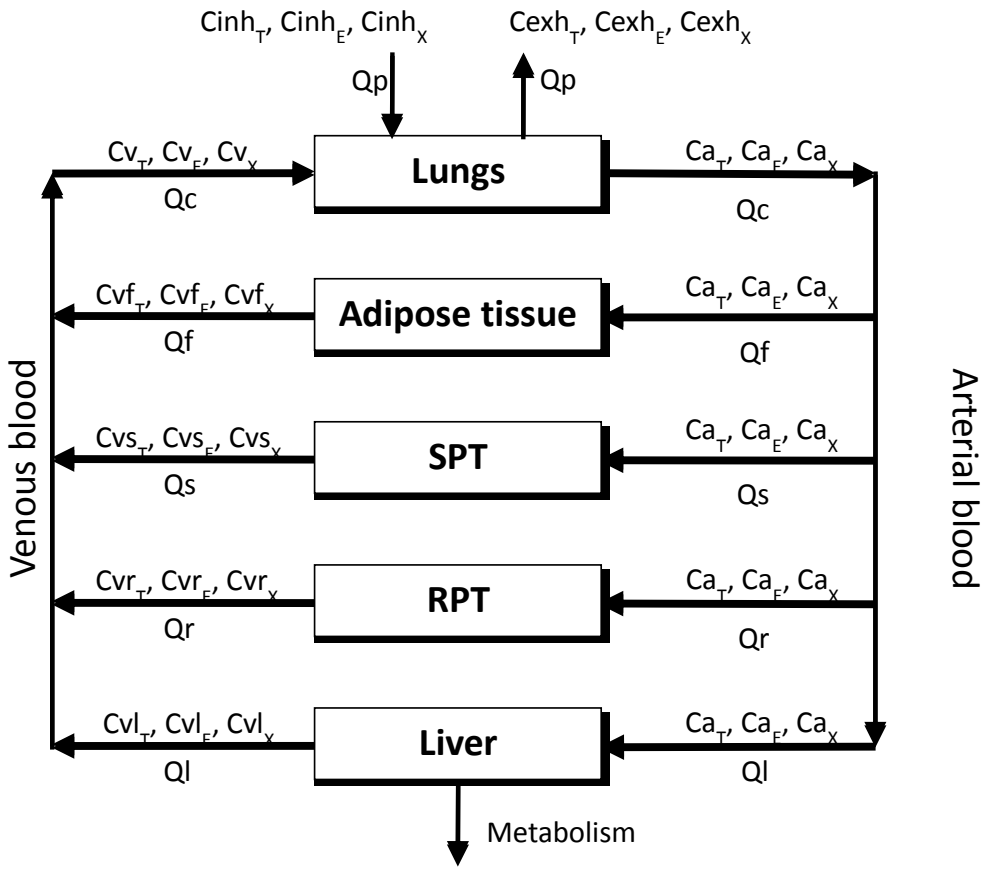
Figure 4. Comparisons of PBPK model simulations (lines) and experimental data (□)of exhaled air concentrations of toluene, ethylbenzene and m-xylene in human volunteers exposed by inhalation to ¼ or 1/8 TLV for 6 h. Simulations were run with existing models

(dotted lines) and with optimized parameters (solid lines). In the case of toluene, the dotted line is for model from Tardif et al (1997) and the solid line is for Haddad et al (1999) model.

Figure 5. Comparisons of PBPK model simulations (lines) and experimental data (□) of urinary excretion of o-cresol, mandelic acid and methyl hippuric acid in human volunteers exposed by inhalation to toluene, ethylbenzene and mandelic at ¼ or 1/8 TLV for 6 h. Simulations were run with existing models (dotted lines) and with optimized parameters (solid lines). In the case of toluene, the dotted line (····) is for model with Km and Vmax values from Haddad et al (1999), the solid line is for the same model with optimized stoichiometric yield and the last simulation (----) is for metabolism by CYP1A2.

2.10 Figures

Figure 1

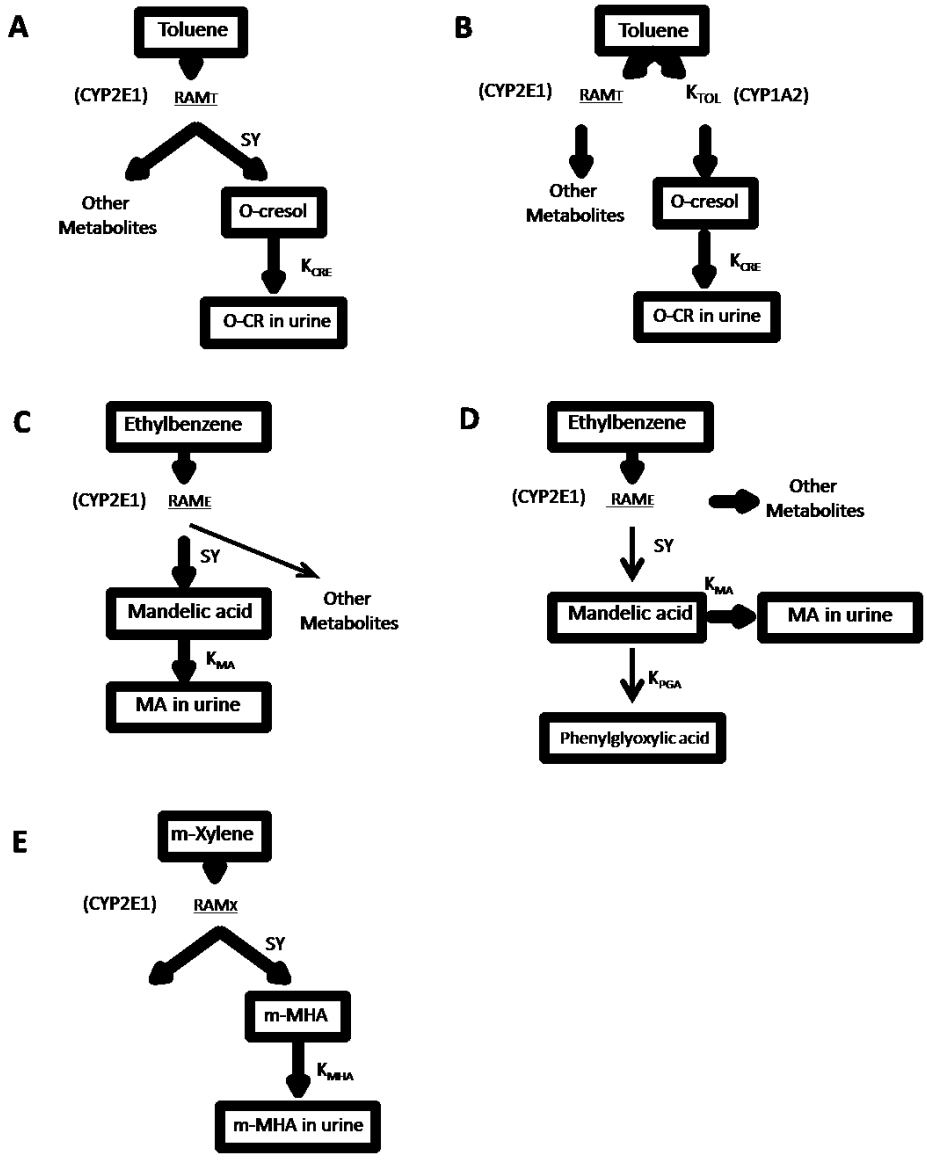


$$RAM_T = \frac{Vmax_T * Cvl_T}{(Km_T + Cvl_T)}$$

$$RAM_X = \frac{Vmax_X * Cvl_X}{(Km_X + Cvl_X)}$$

$$RAM_E = \frac{Vmax_E * Cvl_E}{(Km_E + Cvl_E)}$$

Figure 2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Toluene
(CYP2E1)
RAMT
SY
Other Metabolites
O-cresol
K_CRE
O-CR in urine
B
Toluene
(CYP2E1)
RAMT
K_TOL
(CYP1A2)
Other Metabolites
O-cresol
K_CRE
O-CR in urine
C
Ethylbenzene
(CYP2E1)
RAME
SY
Other Metabolites
Mandelic acid
K_MA
MA in urine
D
Ethylbenzene
(CYP2E1)
RAME
Other Metabolites
SY
K_MA
Mandelic acid
MA in urine
K_PGA
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SY
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K_MHA
m-MHA in urine

Figure 3

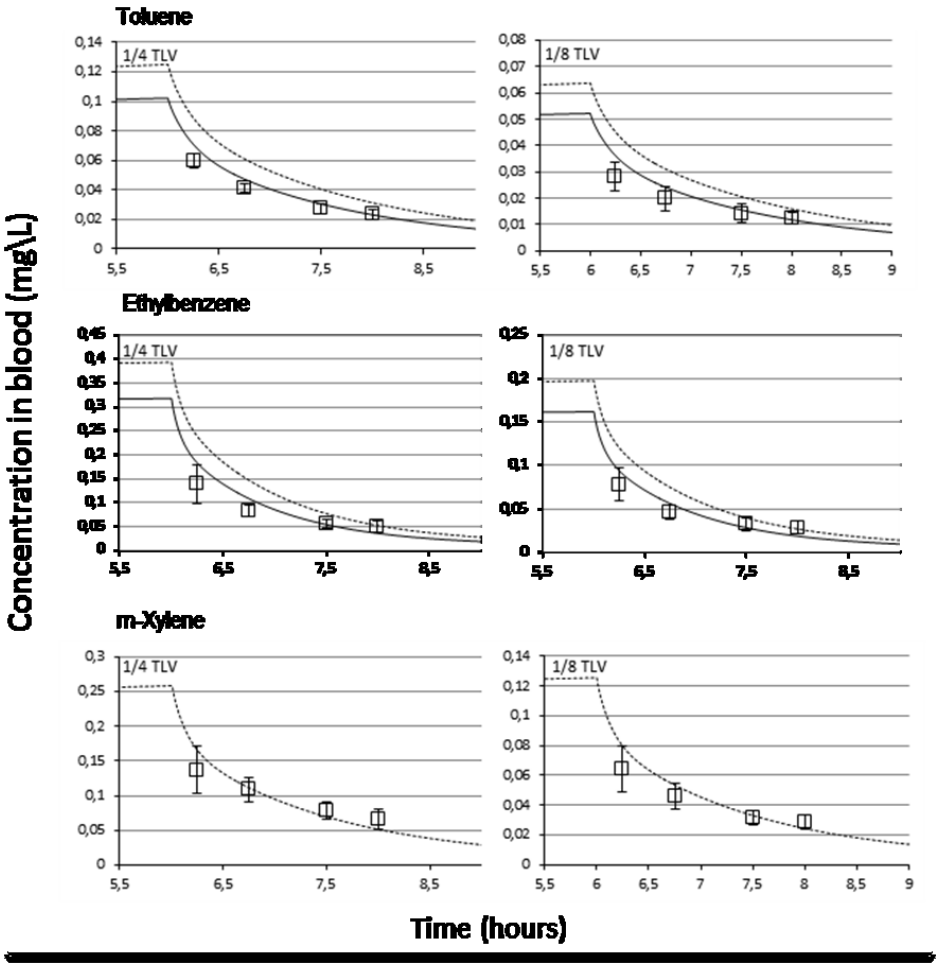
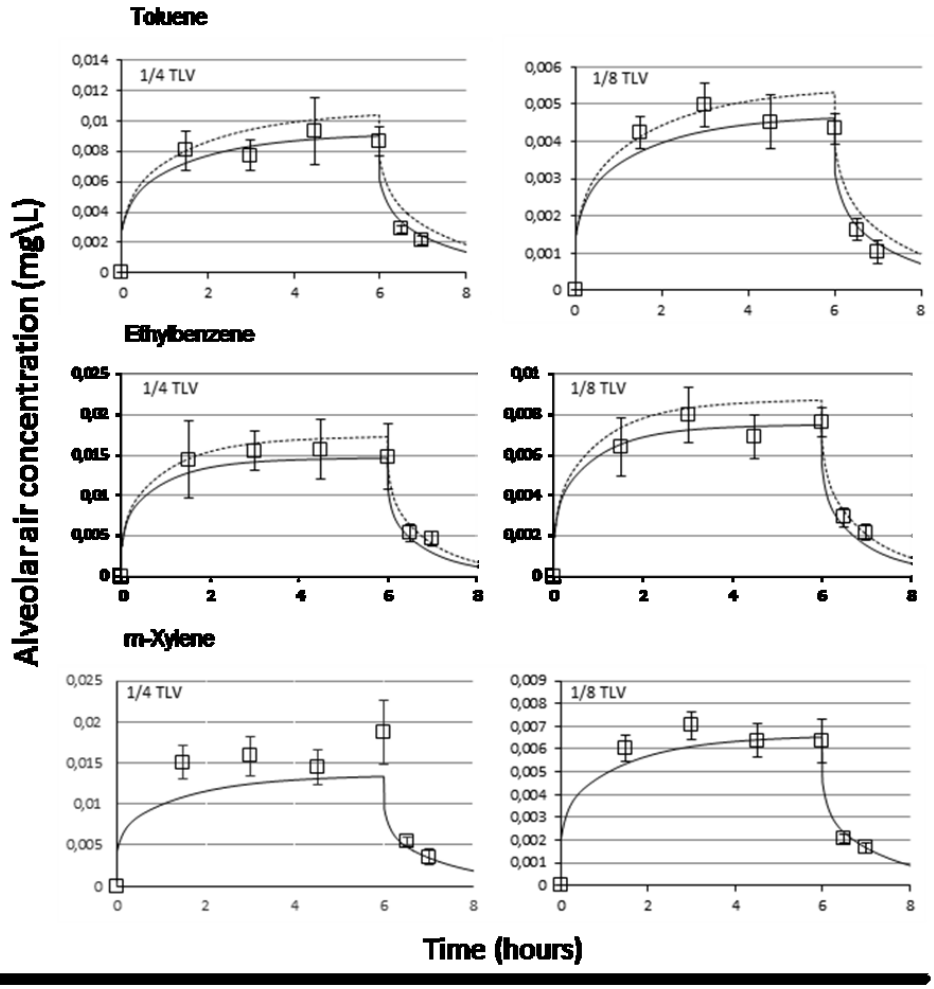

Toluene
1/4 TLV
1/8 TLV
Ethylbenzene
1/4 TLV
1/8 TLV
m-Xylene
1/4 TLV
1/8 TLV
Concentration in blood (mg\L)
Time (hours)

Figure 4


Toluene
1/4 TLV
1/8 TLV
Ethylbenzene
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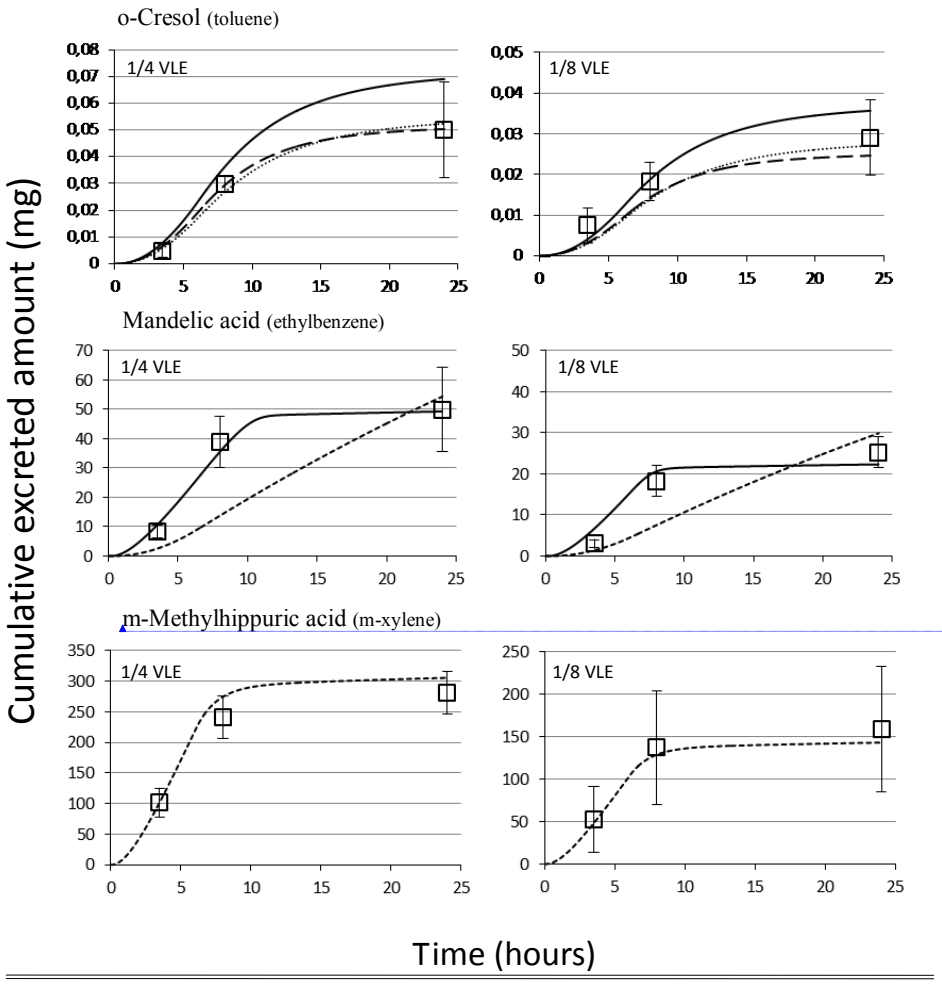
1/8 TLV
m-Xylene
1/4 TLV
1/8 TLV
Alveolar air concentration (mg\L)
Time (hours)

Figure 5



Mis en forme : Anglais (Canada)

Figure 6

2.11 Tables

Table 1. Monitored mass transition and retention time of quantified metabolites.

Chemicals	RT (min)	Monitored *m/z* transitions	Calibration Concentrations interval (µM) [a]	LOQ[b] (mg/L)
Cresol-d8 (IS)	5.083	295 → 181	7.21	NA
o-Cresol	5.11	288 →181	0.2 - 4.06 (R^2=0.999)	0.00189
Mandelic acid-d5 (IS)	7.14	111.8 → 84	781.1	NA
Mandelic acid	7.16	107→79	132-1986 (R^2=0.999)	1.89
m- Methylhippuric acid	9.85	119→ 91	113 – 2255 (R^2=0.999)	1.54

[a] Each calibration curves consisted of 5 different concentrations within the given concentration interval.

[b] Calculated as 10-times the standard error from a series of 10 injections of the lowest standard

Table 2. Physiological parameter values for a standard adult human from Tardif et al. (1997)

Parameters	Symbol	Value
Body weight	BW	Average values measured for volunteers
Cardiac output	Qc	$18*BW^{0.7}$
Alveolar ventilation rate (L/hr/Kg)	Qalv	$18*BW^{0.7}$
Compartment volume (%BW)		
Adipose tissue	Vf	19
Liver	Vl	2.6
Richly perfused tissues	Vr	5
Poorly perfused tissues	Vmp	62
Blood flow to tissue compartment (%Qc)		
Adipose tissue	Qf	5
Liver	Ql	26
Richly perfused tissues	Qr	44
Poorly perfused tissues	Qmp	25

Table 3. Chemical specific parameters

Parameters	Symbol	TOL[a]	EBZ[a]	m-XYL[a]
Partition Coefficients				
Blood :air		18	42.7	46
Liver:air		83.6	83.8	90.9
Adipose tissue :air		1021	1556	1859
Richly perfused tissues :air		83.6	60.3	90.9
Slowly perfused tissues:air		27.7	26	41.9
Metabolic constants				
Maximal rate (mg/hr/kg)	Vmax	4.8	7.3	5.5
		3.45[b]	14.35[c]	-
Affinity constant (mg/L)	Km	0.55	1.39	0.22
		0.134[b]	-	-

[a] Values from Tardif *et al*. (1997)

[b] Values from Haddad et al. (1999)

c Optimized value in 3-step model

Table 4. Parameter values for o-cresol, mandelic acid and m-methylhippuric acid formation and excretion

Urinary Biomarker	Parameters	Symbol	Literature value	Optimized value	Extrapolation
O-cresol	**CYP2E1 model**				
	o-CR excretion constant	K_{CRE}	0.715[a]	-	$BW^{-0.3}$ [b]
	Stoichiometric yield	SY	0.00078[b]	0.00059	
	CYP1A2 model				
	o-CR first order constant (h^{-1}▪kg^{-1})	K_{TOL}	n.a.	0.665	$BW^{-0.3}$
Mandelic acid	**1 step model**				
	MA excretion constant (h^{-1})	K_{MA}	n.a.	0.19	
	Stoichiometric yield	SY_{MA}	0.7[d]		
	3 step model				
	EBZ to MA (mg/hr/kg)	$Vmax_E$	7.3[c]	13.32	$BW^{0.75}$ [c]
	Stoichiometric yield	SY_{MA}	0.9[d]	-	
	MA to PGA (hr^{-1})	K_{PGA}	n.a.	0.265	
	MA excretion constant (h^{-1})	K_{MA}	n.a	0.09	
Methylhippuric acid	MHA excretion constant	K_{MHA}	1.3[e]		
	Stoichiometric yield	SY_{MHA}	0.97[d]		

[a] Baelum *et al* (1985)
[b] Tardif *et al* (2002)
[c] Tardif *et al* (1997)
[d] Engström *et al* (1984)
[e] Riihimaki (1979)

2.12 Appendix

2.12.1 Analytical Methods

Parent compound analyses in exhaled air and chamber air

Concentrations in chamber and exhaled air samples were analyzed for parent compounds by gas chromatography as previously described (Tardif *et al*, 1991). Briefly, air from chamber and Tedlar bags was routed by a pump to an automatic injection system for volatile compounds including an electric valve and a pneumatic injection loop (1mL volume) which was coupled to a Varian CP-3800 gas chromatograph. The column used was an HP1 (30 m x 0.53 mm, 1.0 mm film thickness) (Agilent Tech, Canada). The column initial temperature was 70 °C and ramped at 15°C/min to 160°C and held for 1 min. The temperature of the injection port and detector were set at 150 and 300 °C, respectively. Toluene, ethylbenzene and m-xylene were analyzed trough a flame ionization detector (FID). The retention times of VOCs were 5.955 min for toluene, 7.058 for ethylbenzene and 7.158 min for m-xylene. The calibration was made using gas standards prepared in Tedlar bags (Integra Environmental, Burlington, Canada)

Parent compound analyses in blood

Blood samples were analyzed by modified method described by Blount *et al* (2006) consisting of a isotope dilution solid phase microextaction gas chromatography tandem mass spectrometry (SMP-GC-MS/MS) Briefly 0.5 g of blood were transferred to a pre-weighed SPME headspace vial using a 3 mL pre-cleaned glass syringe and 30.0 µL of internal standard solution were added, the vial was crimped shut and mixed thoroughly. Samples were queued in the Combi-PAL (Varian, Palto Alto, CA) vial tray, cooled to 15 ± 0.5 °C until analysis.

The analytical instrument was a TRACE Ultra Gas Chromatograph-TSQ quantum XLS mass spectrometer (ThermoFisher Scientific, MA). A Cryotrap 915 (ThermoFisher Scientific, Milan, Italy) was used to cryo-focus analytes using approximately 11 cm of base deactivated-guard column (Restek, Chromatographic Specialties, Brockville, ON). The column used was DB-VRX capillary column (0.18 mm x 40 m, 1.0 µm film thickness) (J&W Scientific, Folsom, CA).

During analysis, each sample was transferred to the conditioning/agitator station, held at 40°C. The SPME fiber (75-µm Carboxen-PDMS coated, Supelco, Bellefonte, PA) was inserted into the sample headspace for 6 min while the vial was agitated at 500 rpm. The SPME fiber was then immediately transferred to the GC injection port held at 220°C, and held for a 30 second desorption time. After injection the cryotrap was maintained at -140°C for 1.5 min, and then heated ballistically to 225°C to desorb analytes from the pre-column. The analytical column was maintained at an initial oven temperature of 0°C to further focus the volatile analyte band. The GC oven was held at 0°C for 1.5 min, ramped at 14°C/min to 145°C and finally ramped at 7°C/min to 160°C for a total chromatographic time of 14 min. The carrier gas flow was maintained at a constant rate of 1mL/min. The detector was a TSQ Quantum XLS Mass spectrometer equipped with an electron ionization source. The source temperature was 180°C, and transfer line temperature was 160°C. The MS was operated in selected reaction monitoring (SRM) mode, with Q1 and Q3 peak width set at 0.7 (FWHM) and a cycle time of 0.2 seconds.

Urinary Metabolite analyses

Urine samples were thawed on ice. 1 mL of sample were hydrolyzed with 2 M H_2SO_4 and heating at 100^{o}C for 30 minutes followed by an extractive alkylation as described by Marais et Laurens, 2005 with some modifications to optimize the extraction. TBA solution concentration was increased to 0.4M and sodium phosphate buffer (mono- and dibasic) was adjusted to a pH of 10,5. Derivatized samples with PFB-Br were then analyzed by an Agilent

7890A series GC coupled to a an Agilent 7000 triple quadrupole (MS/MS) mass spectrometer (Agilent Technologies, Palo Alto, CA, USA) and a CTC-CombiPal automatic injector (CTC, Switzerland). Inlet temperature was increased to 300^{o}C to allow better volatilization of derivatized metabolites. A DB-1MS fused-silica capillary column (30m x 250 µm, 0.1µm film tickness) (Agilent Technologies) was used with helium as gas carrier at a flow rate of 1. 3 mL/min. The initial temperature was80^{o}C and held for 1 minute and ramped at 5^{o}C/min to 130^{o}C. Then it ramped at 10^{o}C /min to 180^{o}C. Third ramp rate was 30 ^{o}C/min to 210^{o}C and last ramp rate was 50^{o}C/min to 300^{o}C. Final temperature was held for 10 minutes. Retention time and parameters for MS/MS analyses are presented in table 1. o-Cresol, mandelic acid and m-methylhippuric acid were the monitored metabolites for toluene, ethylbenzene and m-xylene metabolisms respectively. o-Cresol-d^{8} and mandelic-2,3,4,5,6-d^{5} acid were used as internal standard.

Chapitre 3. Évaluation et modélisation de l'impact de coexpositions aux mélanges de COV sur les biomarqueurs urinaires

3. Evaluation and modeling of the impact of co-exposures to VOC mixtures on urinary biomarkers

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3.1 Abstract

Urinary biomarkers are widely used among biomonitoring studies because of their accessibility. Chloroform, ethylbenzene, toluene and m-xylene are common chemicals that are often found together. Although interactions occurring between the three last VOC are well known, no information exists on possible interaction between these chemicals and chloroform at the level of kinetics of parent compound or urinary biomarkers. The objective of this study was therefore to study the possible interactions between these compounds in human volunteers. Five male volunteers were exposed by inhalation for 6h to single, binary and quaternary mixtures that included chloroform. Air concentrations of volatiles were set at ¼ or 1/8 of the TLV. Exhaled air and blood samples were collected and analyzed for parent compound concentrations. Urinary biomarkers (o-cresol, mandelic and m-methylhippuric acids) were quantified in urine samples. Published PBPK model for chloroform was used and a Vmax of 3.4 mg/h/kg was optimized to provide a better fit with blood data. Adapted PBPK models from the previous study were used for parent compounds and urinary biomarkers for TEX. Binary exposures with chloroform resulted in no significant interactions. Experimental data for quaternary exposures were well predicted by PBPK models using published Ki for competitive inhibition or not, thus demonstrating that no significant interaction occur at levels used in this study. PBPK models for urinary biomarkers proved to be a good tool in quantifying exposure to VOC.

Keywords: Mixtures, PBPK modeling, VOC, urinary biomarkers

3.2. Introduction

Simultaneous exposures to multiple chemicals the environment and in occupational settings are the norm rather than the exception. Current biomonitoring approaches are usually based on simple correlations between exposure and metabolites levels without any consideration for potential interactions occurring between coexposed chemicals and their effects on levels of parent compounds and/or their metabolites.

Toluene, ethylbenzene and m-xylene are volatile organic compounds (VOC) for which co-exposure by inhalation is common due to their large application in common fields and products. Chloroform has also a widespread distribution since it is the major byproduct formed from water chlorination and is highly volatile. Although many studies, mostly based on Corley *et al* (1990) model, were conducted on its kinetics, few were made regarding co-exposure with other VOC. Because chloroform is also mainly metabolized by the same enzyme than the previous three VOC, it is reasonable to assume that metabolic competition would occur and thus result in different levels of the parent compounds and their metabolites in the different biomonitored matrices.

According to the literature, information on binary exposures should be sufficient to predict the behavior of each chemical in a mixture (Tardif *et al* 1997, Haddad *et al.* 1999, 2000). Since information was already available for interactions occurring between toluene, ethylbenzene and m-xylene, the first objective of this study was to collect human toxicokinetic data on these aromatic solvent and their urinary biomarkers from binary exposures with chloroform. Additionally we aimed to get insights on the mechanisms governing observed interactions and describe them with physiologically based toxicokinetic (PBTK) modeling. Finally using information from the binary interactions, the hypothesized binary interactions will be tested by simulating quaternary mixture exposures and comparing to exposure data to such mixtures obtained in this study.

3.3 Material and methods

3.3.1 Volunteer exposures and chemical analyses

3.3.1.1 Material

Toluene (99.8% purity), ethylbenzene (99% purity) and m-xylene (≥99% purity) were purchased from Sigma Aldrich. o-cresol (≥99% purity), m-methylhippuric acids (98% purity), mandelic acid (99% purity), hippuric acid (98% purity), o-cresol-d^8, 2,3,4,5,6-pentafluorobenzyl bromide (PFB-Br) (99% purity), tetrabutylammonium hydrogensulphate (TBA), methanol (≥99% purity), dichloromethane (≥99% purity), sodium hydroxide (1M), sulfuric acid (0,5 M), $NaH_2PO_4.H_2O$ were purchased from Sigma Aldrich. Mandelic-2,3,4,5,6-d^5 acid was purchased from CDN Isotope Inc. (Pointe-Claire, Canada). Toluene-$^{13}C_6$, Ethylbenzene-$^{13}C_6$ and m-xylene-^{13}-C_2 were purchased from Cambridge Isotope Laboratories Inc (Andover, MA).

3.3.1.2 Volunteers

The project was approved by the Ethics Committee of the Université de Montréal and Health Canada. Each volunteer signed an informed consent and provided medical information pertinent to the study. They were told not to drink alcohol 24 h before and following the beginning of exposures. Volunteers were males aged between 18 and 35, weighting between 58 and 100 kg, non-smokers and with no known recent history of exposure to VOCs.

3.3.1.3 Exposure chamber

As previously described by Tardif *et al.* (1991), the human exposure chamber is a 18 m^3 room connected to an air generator which is located at the inhalation laboratory of the Department of Environmental and Occupational Health of the Université de Montréal. To volatilize COV in the atmosphere of the room, solvents were pumped, using an HPLC pump

(Varian 2510), in a round-bottom flask located upstream of the chamber inlet. A stream of pressurized air then volatizes the chemicals. The volatilized COVs are then distributed through 16 diffusers in the chamber ceiling.

3.3.1.4 Exposures

Groups of 5 Volunteers were exposed to chloroform alone, in binary and quaternary mixtures with toluene, ethylbenzene and m-xylene (Table 1). At every exposure session, the volunteers entered the inhalation chamber only when the concentration in the room reached steady state. The exposure concentrations were ¼ and 1/8 of TLV (ACGIH, 1995-1996), hence the highest level of exposure during quaternary mixture did not exceed a Hazard Index (HI or Rm) of one (i.e., HI= ¼ $TLV_{toluene}$+¼ $TLV_{m\text{-}xylene}$ +¼ $TLV_{ethylbenzene}$ +¼ $TLV_{chgloroform}$ = 1) according to ACGIH mixture formula (ACGIH, 1963).TLVs from 1995-1996 were used for comparison with previously reported data (Tardif *et al*, 1997). Exposures lasted 6 h/day with at least one-week interval between each of them. The TLV for these chemicals are : 50 ppm for toluene,100 ppm for ethylbenzene and m-xylene, and 10 ppm for chloroform. Because water is a major source of exposure to chloroform, volunteers were also given drinking water containing 90 ug/L (±5 ug/L) of chloroform in two exposures while exposed by inhalation to toluene, ethylbenzene and m-xylene. A total of 500 ml of water was given to each volunteer, more specifically 125 ml at four time points (same as exhaled air sampling times) during the 6 hours inhalation exposure.

Exhaled air samples were collected in 3L Tedlar bags during exposure (0, 1.5, 3, 4.5 and 6 h) and after (6.5 and 7h). One blood sample was collected with a catheter before exposure to measure background exposure and other samples were collected after the end of exposure (15, 45, 90 and 120 minutes after). Blood samples were then kept refrigerated until analysis. Urine was collected before, during (3.5 h) and after the exposure (8, 15 and 24h after start of exposure) and then stored at -20^{o}C for further analysis.

3.3.1.5 Analytical Methods

3.3.1.5.1 Parent compound analyses in exhaled air and chamber air

The VOCs in air of the chamber were monitored with 10 minutes intervals using a Varian CP-3800 with FID and ECD detectors coupled with an automatic headspace injection system and an electric valve and a pneumatic injection loop (1mL volume). The column used was an HP1 (30m x 0.53 mm; 1.0 mm film thickness) (Agilent Tech, Canada). Settings for GC analyses are the same as described previously (Marchand et al, 2014). The samples were always taken at one place in the inhalation chamber and not in the breathing zone of the volunteers. Additionally, an infrared spectrophotometer (MIRan) was connected to the inhalation chamber to provide continuous measurements. A mean variation of 5% was observed for chamber concentrations (considering all exposures). Exhaled air samples collected in Tedlar bags were measured by the gas chromatograph with the same method as for the chamber.

3.3.1.5.2 Parent compound analyses in blood

Blood samples were analyzed for parent compound concentrations using a modified method from Blount et al. (2006) as previously described (Marchand et al, 2014).The method consists of an isotope dilution approach combined with solid phase microextraction gas chromatography tandem mass spectrometry (SMP-GC-MS/MS).

3.3.1.5.3 Urinary Metabolite analyses

As described in the previous article (Marchand et al 2014), urinary biomarkers for toluene, ethylbenzene and m-xylene were analyzed on a method derived from the method of Marais and Laurens (2005) for analysis by GC-MS/MS. The method consisted of an acidic deconjugation followed by an extractive alkylation with PFB-Br.

3.3.1.5.4 Statistics

Student t test were performed where single exposures were compared with multiple chemical exposures. Because volunteers were not always the same between exposures, spaghetti diagrams (see Figures A1 and A2 in appendix) were made for each volunteer in order to confirm potential effect of exposure on blood concentrations or total excreted metabolites (see Table A1 in Appendix).

3.3.2 PBPK modeling of parent compounds and metabolites

3.3.2.1 Model representation

Parent compound

The PBTK model for each parent compound is composed of four compartments, namely richly perfused tissues, slowly perfused tissues, adipose tissue and liver which are interconnected by systemic circulation and gas exchange in the lung (Figure 1). Metabolism for each chemical is limited to the liver and described by Vmax (mg/h) and Km (mg/L). The four VOC PBTK models are linked through metabolism by an inhibition constant (Ki) at the binary level assuming a competitive inhibition mechanism (Haddad et al 1999).

Urinary biomarkers

Formation and excretion of urinary biomarkers are represented (Figure 2) as described in the previous article for single chemical exposures (Marchand et al, 2014). Stoichiometric yields or first order constant in the case of o-cresol (CYP1A2) are being used with excretion constants for modeling metabolite excretion. Ethylbenzene metabolism is described by a 2 steps model with a first order rate constant for phenylglyoxylic acid formation.

3.3.2.2 Model parametrization

Parameters for chloroform were taken from Haddad et al (2006) (Table 2). Those for the three others solvents were taken from Tardif et al (1997) except for toluene for which Vmax and Km values were taken from Haddad et al (1999) as determined in the previous article (Marchand et al, 2014). Ki values for binary combination of toluene, ethylbenzene and m-xylene were taken from Tardif et al (1997) (Table 5). All parameters for urinary biomarkers were the same than those in the previous article (Marchand et al, 2014). Those values are reported in Table 4.

3.3.2.3 Model calibration and simulations

Parent compounds concentrations in blood and exhaled air resulting from binary and quaternary exposures were compared with predictions from PBTK models optimized with single chemical exposures first assuming no interaction. Ki values were then added to models to see if predictions would be closer to experimental data

To describe kinetics of urinary biomarkers, inhibition was first limited to the first step of metabolism for each chemical. Both ways for o-cresol formation (metabolism by CYP2E1 or by CYP1A2) were tested. Results for o-cresol excretion were also compared with previous reported stoichiometric yield factor of 0,00078.

3.4 Results

3.4.1 Single exposure to chloroform

The use of Km and Vmax values from Corley et al (1990) (reported in the model of Haddad et al. 2006) led to underestimation of chloroform blood levels, especially regarding data collected 90 and 120 minutes after end of exposure. Optimization of Vmax (Table 5) gave a value of 3,4 mg/h/kg which represent about 26% of the initial value. The new Vmax value

gprovided a good fit with experimental data at both levels of exposure (Figure 3). Concentrations in alveolar air were well predicted by the model using both Vmax values.

3.4.2 Binary mixtures with chloroform

An inhibition occurring during coexposure of two chemicals would generate an increase in parent compound levels in the body compared to single chemical exposure. Comparison of experimental data for single exposures with experimental data for binary mixtures with chloroform yielded no significant increase in chloroform levels in blood nor in exhaled air for co-exposed chemicals (Figures 4 and 5). Surprisingly, some statistically significant decreases were observed in blood levels while no effects were noticed for exhaled air samples for any binary mixtures (Figures 4 and 5). This was observed in the co-exposure to chloroform (1.25 ppm) and m-xylene (25 ppm) where a decrease in parent compound levels in blood for both chemicals was noticed at all sampling times (Figure 4). Co-exposure to chloroform (1.25 ppm) with ethylbenzene (25 ppm) also resulted in a decrease of chloroform blood concentrations but no changes were observed for ethylbenzene blood levels. Because no inhibition was observed, no Ki values were optimized at the level of CYP2E1 metabolism between chloroform and the aromatic solvents.

Regarding urinary biomarkers, no statistically significant changes were observed for o-cresol, mandelic and m-methylhippuric acids excretion (Figure 6). The total excreted amount of o-cresol was well predicted by the model developed in the previous study (Marchand et al, 2014). Mandelic acid was slightly underestimated by the model for the 24h data. In opposite, prediction for total excreted amount of m-methylhippuric acid was slightly over the standard deviation range.

3.4.3 Quaternary mixtures

Comparison of experimental data from single exposures with data from quaternary exposures by inhalation resulted in blood level decreases in parent compound only for the first two sampling times for toluene (1/4 TLV) and for all sampling times for m-xylene (1/8

TLV) (Figure 7). Similarly, no variations for parent compound in exhaled air samples were observed (Figure 8). Total excreted amount of m-methylhippuric acid showed some significant decreases compared to single exposure experimental data (i.e. 24% and 29% decrease for 1/4 and 1/8 TLV exposure level respectively) (Figure 9).

The total intake of chloroform via drinking water during exposures 17-18 was between 40.32 and 45.36 µg while more than 9.5 mg of chloroform was absorbed during the 6 hours of inhalation at 1.25 ppm of chloroform (exposure 2). Because the amount of chloroform in drinking water is only a small fraction of that from inhalation exposure, last two exposures with chloroform in drinking water could be considered like ternary mixtures of TEX. In those ''ternary'' exposures, again no differences for parent compound in exhaled air were observed (Figure 8). However, some variations in blood levels were noticed (Figure 7). Only first two sampling times (15 and 45 min after end of exposure) for toluene in blood showed a significant decrease for TEX exposure compared to single exposure at the same level (12.5 ppm). Exposure to the lower level of TEX (1/8 TLV) did not result in any variation for toluene. Blood concentrations of m-xylene showed increases of 18-34% for the higher exposure level to TEX (1/4 TLV) and 31-39% for the lower level of exposure to the same mixture (1/8 TLV). However, only results for exposure at 1/8 of the TLV are statistically significant. That increase in blood level is similar to prediction of the model assuming competitive inhibition, but the model without interactions could predict experimental data as well. No differences in alveolar air concentrations were noticed and the model predicted adequately the experimental data for each chemical.

Total excreted amount of o-cresol were 47 and 61% higher in exposures 17 and 18 than for single exposures 3 and 4. An increase is also observed for mandelic acid (i.e. 34% and 51% increase compared to single exposure at 25 and 12.5 ppm respectively). Except for o-cresol (1/4 TLV), model predictions for total excreted amount of o-cresol or mandelic acid were under the standard deviation range. In the previous study (Marchand et al, 2014), a model for o-cresol formation via CYP1A2 was adapted. The model was tested with competitive

inhibition (Figure 10). No variations in methylhippuric acid levels were observed and model adequately predicted the experimental data throughout the 24h period.

3.5 Discussion

This study allowed i) to collect experimental kinetic data on toluene, m-xylene and ethylbenzene and their urinary biomarkers following co-exposures with chloroform in binary and quaternary mixtures, ii) to evaluate if interactions occurred between co-exposed chemicals, iii) and to model observed interactions using a PBPK approach. Experimental data from mixture exposures were compared to those from single exposures as well as with model predictions in order to evaluate if co-exposures to those COV could results in error of interpretation regarding biomonitoring

Even if exhaled air has been reported to be a good way for biomonitoring of COV exposure, usual techniques to collect it remain imprecise and can lead to greater variation within results. Therefore, exhaled air would not be the preferred way to investigate small variations in the metabolism of some COV. In this study, no significant variations in parent compound levels in exhaled air could be observed (Figures 5 and 8).

Blood is another good biological matrix for COV exposure evaluation, but more invasive than alveolar air or urine. Although no shifts in blood levels for parent compounds were observed for ethylbenzene, concentrations of toluene showed a decrease of 29% in the presence of ethylbenzene and m-xylene (chloroform in water), but only for the higher exposure levels and only for the first two sampling times. Results for m-xylene blood levels when co-exposed with chloroform were also significantly lower only for the higher level of exposure (i.e. 25 ppm of m-xylene with 1.25 ppm of chloroform). If the decreased blood levels observed for m-xylene were due to a metabolic induction by chloroform, it would be expected that exposure to a higher dose of chloroform would lead to a similar or greater decrease in m-xylene blood levels, which was not observed. Furthermore, one should note

the fact that volunteers were not the same for all exposures and it may have contributed to greater variability in the results. Therefore, we could consider that observed decreased xylene levels in blood are simply due to variability across volunteers and may not be the result of any interaction. In a study where men were exposed simultaneously for 7 hours to TEX (Tardif *et al*, 1997), at concentrations little higher than ours (17, 33 and 33 ppm respectively), only m-xylene levels in blood showed an increase compared to single exposure. Exposure to TEX mixture in the present study also resulted in increased m-xylene blood levels, although only results for the lower level of exposure showed interactions that were statistically significant (Figure 7). A similar increase in m-xylene blood levels (19-27%) was also observed by Tardif *et al* (1997).

O-cresol is thought to be the best metabolite to monitor in urine at low level exposures to toluene (Tardif *et al,* 1998). Results for binary and quaternary exposures support that fact since model could predict the experimental data (Figures 6 and 9). In the previous study (Marchand *et al,* 2014) it was also proposed that formation of o-cresol occur via the CYP1A2. As shown in Figure 10, that model could lead to an increase in o-cresol levels if inhibition occurs. According to toluene blood levels for TEX exposures, inhibition is not likely to be sufficient to explain the observed increase in o-cresol levels. Another simulation in Figure 10 represents the prediction using the former stoichiometric yield reported by Tardif *et al*. (2002) and does allow a better fit with experimental data. This suggests that observed increases for o-cresol could be partly if not all due to inter-individual variability between volunteers from the different exposures and that modeling of o-cresol as a fraction of CYP2E1 metabolism seems to give more accurate predictions.

Hippuric acid is also often mentioned as a urinary biomarker for toluene (Tardif *et al,* 1998). Because of high endogenous levels of hippuric acid are measured with our analytical method (not shown), this urinary biomarker could not adequately quantitatively reflect toluene exposure levels. Hence, it would not be possible to observe any interactions using this biomarker.

Since 2,4-dimethylphenol (DMP), the second metabolite of m-xylene, is thought to be only metabolized by CYP1A2 (Tassaneeyakul *et al*, 1996) like o-cresol, it was quantified in urine samples from the exposures. The ratio of m-MHA:2,4-DMP stayed the same in all exposures (data not shown). Those observations are supported by *in vitro* studies regarding substrate dynamics using m-xylene and CYP2E1 that has shown that this isoform can produce m-methylhippuric acid and 2,4-dimethylphenol in proportions similar to those observed in human inhalation studies (Harrelson *et al*, 2007; Harrelson *et al*, 2007). Also, a human study on coexposure of m-xylene and ethylbenzene revealed a constant m-MHA:DMP ratio between the different schemes of exposure (single or binary mixture, different concentrations) while total urinary metabolites for m-xylene varied (Engström *et al*, 1984). Therefore, the use of a stoichiometric yield constant is more relevant in modeling urinary metabolites for m-xylene than a description of alternate metabolic pathways.

Compared to m-xylene blood levels for TEX exposures, m-methylhippuric acid levels in urine for both levels of exposure were very well predicted by the model assuming no interactions (Figure 9), suggesting that no inhibition of CYP2E1 occurs. However, modeling using Ki from Tardif et al (1997) also resulted in good fits with experimental data. Competitive inhibition may occur, but is simply not observed because of the low levels of exposure used in this study.

In the case of mandelic acid excretion, increases observed for TEX exposures may also be related to interindividual variability for stoichiometric yield for mandelic acid. Otherwise, it would be difficult to explain why no increases in mandelic acid excretion were observed for CTEX exposures. The only way to produce more mandelic acid without affecting blood levels is by diminishing phenylglyoxylic acid. Since that metabolite is formed by ADH as for m-methylhippuric acid, levels of the latter metabolite should be affected too, which was not observed in this study.

3.6 Conclusion

In conclusion, inhibition between co-exposed chemicals at levels equal or lower than ¼ of the TLV is not likely to occur or to be detected. Therefore, modeling of TEX interactions with competitive inhibition for CYP2E1 is sufficient to describe urinary biomarkers levels. Moreover, o-cresol, mandelic acid and m-methylhippuric acid have proved to be good urinary biomarkers for low level exposures to toluene, ethylbenzene and m-xylene using PBPK modeling. These models will be useful in the context of biomonitoring for environmental exposure. They will be useful to estimate exposure to these solvents in context of large scale exposure biomonitoring studies such as the Canadian Health Measures Survey (CHMS).

3.7 Acknowlegments

This research project was supported by Health Canada. The authors would like to thank Ginette Charest-Tardif, Hayet Belmeskine, Jeromy Harvie and Zhiyun Jin for their technical support, Annabelle Espurt for her assistance in blood sampling, Josée Dumas-Campagna for her assistance during sample collections and finally but not least all the volunteers for their precious help.

3.8 References

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3.9 Figure captions

Figure 1. Conceptual representation of the physiologically based toxicokinetic model for chloroform (C), toluene (T), ethylbenzene (E) and m-xylene (X). SPT and RPT are for slowly and rapidly perfused tissues. This representation assumed competitive inhibition beween all four chemicals. Qp and Qc refers to alveolar ventilation rate and cardiac output respectively while Qf, Qs, Qr and Ql refer to blood flows to each compartment. Cv and Ca are for venous and arterial blood concentration and Cinh and Cexh are for inhaled and exhaled air concentration. The rate of the amount metabolized (RAM) is described by the maximal velocity of of the metabolism (Vmax) and Michaëlis affinity constant (Km). Metabolism of each chemical is linked by inhibition constants (Ki).

Figure 2. Conceptual representation of (A) o-cresol formation by CYP2E1 using a stoichiometric yield (SY) and an excretion constant (K_{CRE}). (B) o-Cresol formation by CYP1A2 using a first order rate constant KTOL and an excretion rate constant. (C) Representation of a 3 steps model for mandelic acid formation including a first order rate limiting step, formation of phenylglyoxilic acid from mandelic acid.and an excretion constant (K_{MA}).(D) Representation of m-methylhippuric acid excretion using a stoichiometric yield and an excretion constant (K_{MHA}). Vmax, Km and Cvl are the maximal velocity of the metabolism, the Michaelis-Menten affinity constant and venous concentration for liver respectively.

Figure 3. Comparisons of PBPK model simulations (lines) and experimental data (◯) of blood and exhaled air concentrations of chloroform in human volunteers exposed by inhalation to ¼ or 1/8 TLV for 6 h. Simulations were run with existing models (dotted lines) and with optimized parameters (solid lines).

Figure 4. Comparisons of PBPK model simulations assuming no interactions (lines) and experimental data of blood concentrations of chloroform, toluene, ethylbenzene and m-xylene from single (◯) and binary inhalation exposures (other symbols) in human volunteers

exposed to ¼ or 1/8 TLV for 6 h. Symbols for chloroform refer to coexposure with toluene (Δ), ethylbenzene (□) and m-xylene (×). *p<0.05

Figure 5. Comparisons of PBPK model simulations assuming no interactions (lines) and experimental data of alveolar air concentrations of chloroform, toluene, ethylbenzene and m-xylene following single (○) or binary inhalation exposures (other symbols) in human volunteers to ¼ or 1/8 TLV for 6 h. Symbols for chloroform refer to coexposure with toluene (Δ), ethylbenzene (□) and m-xylene (×). *p<0.05

Figure 6. Comparisons of PBPK model simulations assuming no interactions (lines) and experimental data of urinary metabolites concentrations of o-cresol, mandelic acid and m-methylhippuric acid from human volunteers exposed to toluene, ethylbenzene or m-xylene alone (○) or binary exposures with chloroform (squares) at ¼ or 1/8 TLV for 6 h. *p<0.05

Figure 7. Comparisons of PBPK model simulations (lines) and experimental data of blood concentrations of chloroform, toluene, ethylbenzene and m-xylene in human volunteers exposed to TEX (Δ) or CTEX (□) mixture by inhalation to ¼ or 1/8 TLV for 6 h (expositions 15 à 18). Simulations were run with (dotted lines) and without (solid lines) competitive inhition. Experimental data for single exposure (○) were added for comparison.*p<0.05.

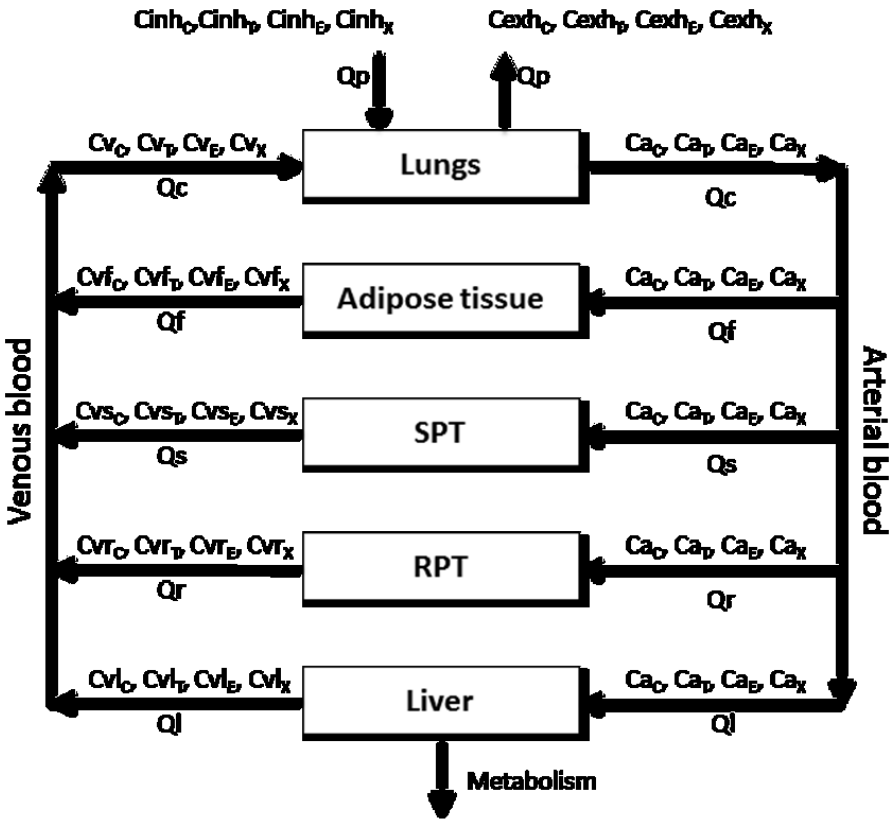
Figure 8. Comparisons of PBPK model simulations (lines) and experimental data of alveolar air concentrations of chloroform, toluene, ethylbenzene and m-xylene in human volunteers exposed to TEX (Δ) or CTEX (□) mixture by inhalation to ¼ or 1/8 TLV for 6 h (expositions 15 à 18). Simulations were run with (dotted lines) and without (solid lines) competitive inhition. Experimental data for single exposure (○) were added for comparison.

Figure 9. Comparisons of PBPK model simulations (lines) and experimental data (□) of urinary metabolites concentrations of o-cresol, mandelic acid and m-methylhippuric acid in human volunteers exposed to TEX (Δ) or CTEX (□) mixture by inhalation to ¼ or 1/8 TLV for 6 h (expositions 15 à 18). Simulations were run with (dotted lines) and without (solid lines) competitive inhition. Experimental data for single exposure (○) were added for comparison. *p<0.05.

Figure 10. Comparison of the different hypothesis for o-cresol formation following TEX exposure (Δ) by inhalation to ¼ or 1/8 TLV for 6h; CYP2E1 without inhibition (solid line), CYP1A2 with inhibition toward CYP2E1 (---) and CYP2E1 without inhibition using former stoichiometric yield of 0,00078 (▪▪▪).

3.10 Figures

Figure 1



$$RAM_C = \frac{Vmax_C \times Cvl_C}{Km_C\left(1+\frac{Cvl_T}{Ki_{TC}}+\frac{Cvl_E}{Ki_{EC}}+\frac{Cvl_X}{Ki_{XC}}\right)+Cvl_C}$$

$$RAM_T = \frac{Vmax_T \times Cvl_T}{Km_T\left(1+\frac{Cvl_C}{Ki_{CT}}+\frac{Cvl_E}{Ki_{ET}}+\frac{Cvl_X}{Ki_{XT}}\right)+Cvl_T}$$

$$RAM_E = \frac{Vmax_E \times Cvl_E}{Km_E\left(1+\frac{Cvl_C}{Ki_{CE}}+\frac{Cvl_T}{Ki_{TE}}+\frac{Cvl_X}{Ki_{XE}}\right)+Cvl_E}$$

$$RAM_X = \frac{Vmax_X \times Cvl_X}{Km_X\left(1+\frac{Cvl_C}{Ki_{CX}}+\frac{Cvl_T}{Ki_{TX}}+\frac{Cvl_E}{Ki_{EX}}\right)+Cvl_X}$$

Figure 2

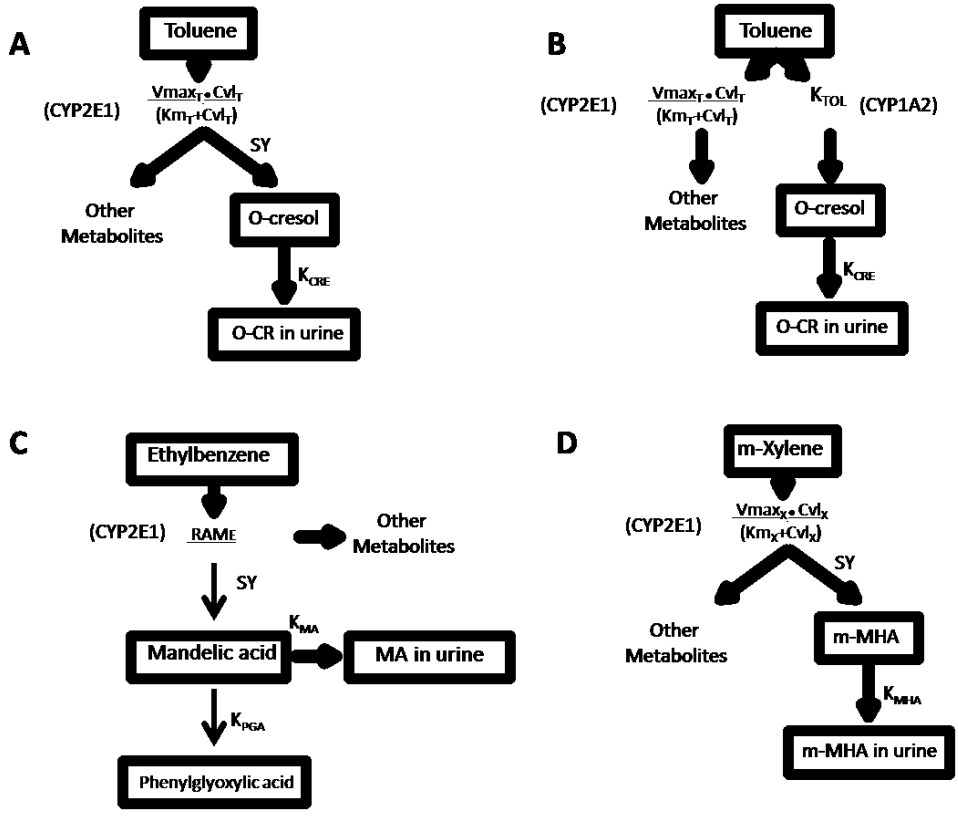

A
Toluene
(CYP2E1)
$\frac{Vmax_T \cdot Cvl_T}{(Km_T+Cvl_T)}$
SY
Other Metabolites
O-cresol
K_{CRE}
O-CR in urine
B
Toluene
(CYP2E1)
$\frac{Vmax_T \cdot Cvl_T}{(Km_T+Cvl_T)}$
K_{TOL}
(CYP1A2)
Other Metabolites
O-cresol
K_{CRE}
O-CR in urine
C
Ethylbenzene
(CYP2E1)
RAME
Other Metabolites
SY
K_{MA}
Mandelic acid
MA in urine
K_{PGA}
Phenylglyoxylic acid
D
m-Xylene
(CYP2E1)
$\frac{Vmax_X \cdot Cvl_X}{(Km_X+Cvl_X)}$
SY
Other Metabolites
m-MHA
K_{MHA}
m-MHA in urine

Figure 3

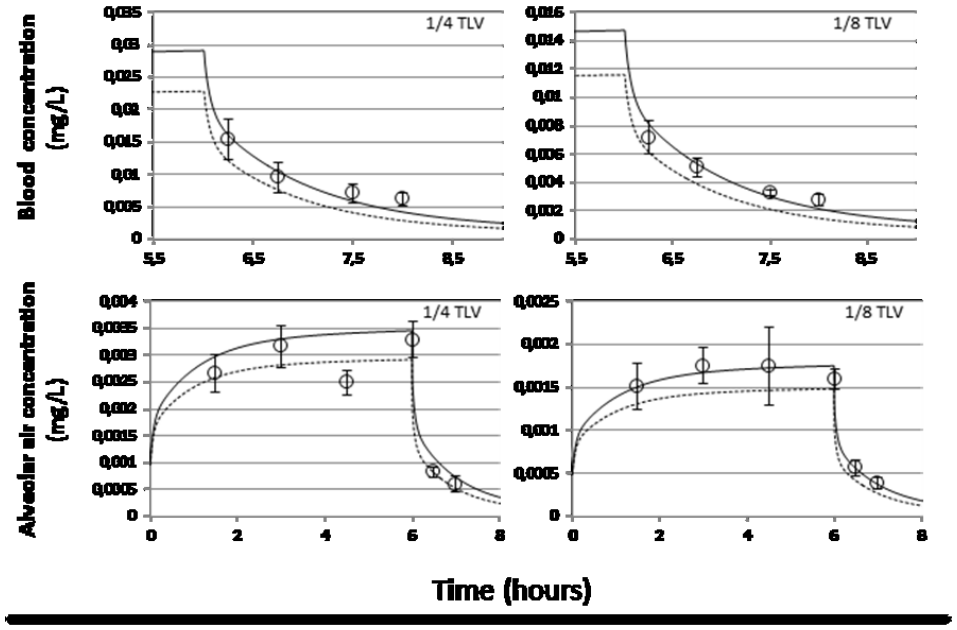

Blood concentration (mg/L)
1/4 TLV
1/8 TLV
Alveolar air concentration (mg/L)
1/4 TLV
1/8 TLV
Time (hours)

Figure 4

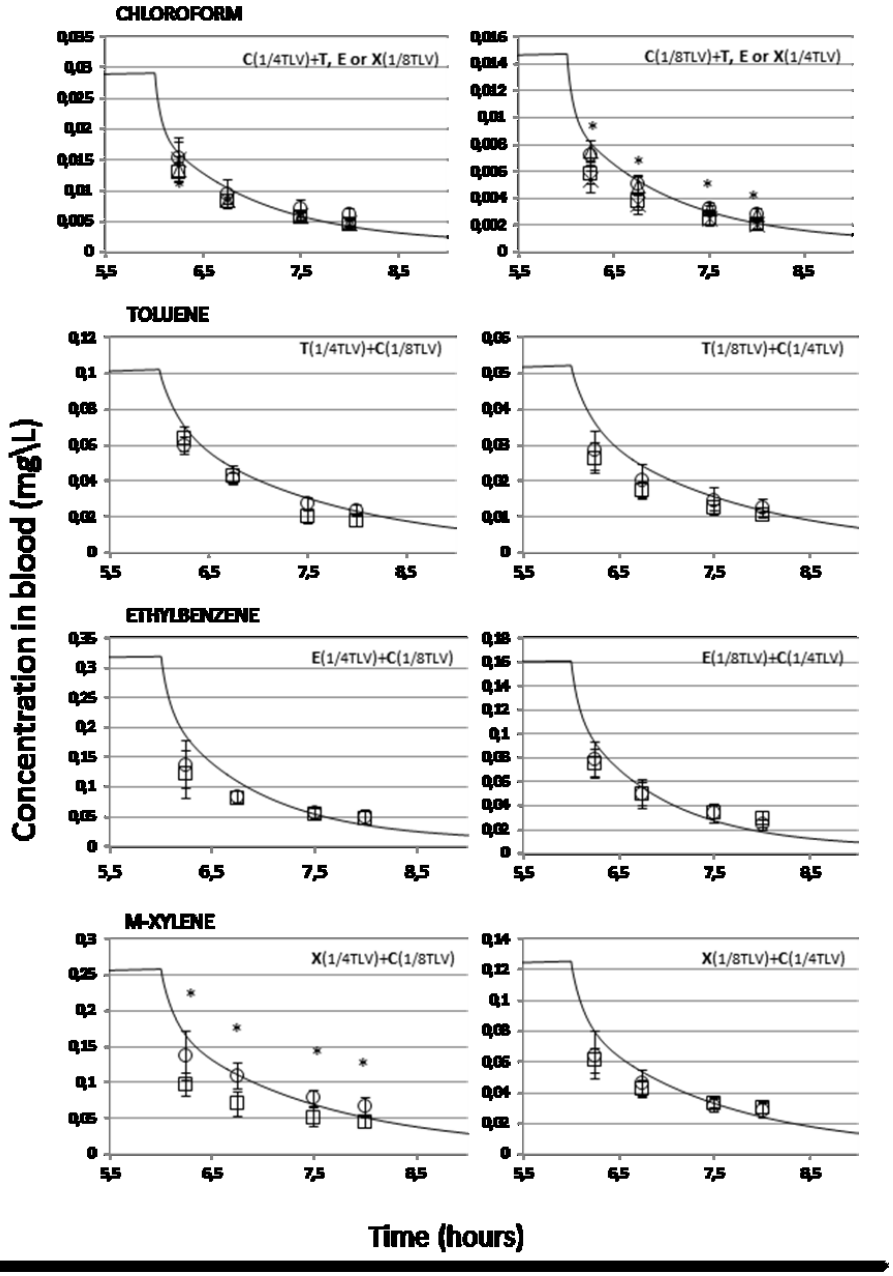

CHLOROFORM
C(1/4TLV)+T, E or X(1/8TLV)
C(1/8TLV)+T, E or X(1/4TLV)
TOLUENE
T(1/4TLV)+C(1/8TLV)
T(1/8TLV)+C(1/4TLV)
ETHYLBENZENE
E(1/4TLV)+C(1/8TLV)
E(1/8TLV)+C(1/4TLV)
M-XYLENE
X(1/4TLV)+C(1/8TLV)
X(1/8TLV)+C(1/4TLV)
Concentration in blood (mg\L)
Time (hours)

Figure 5

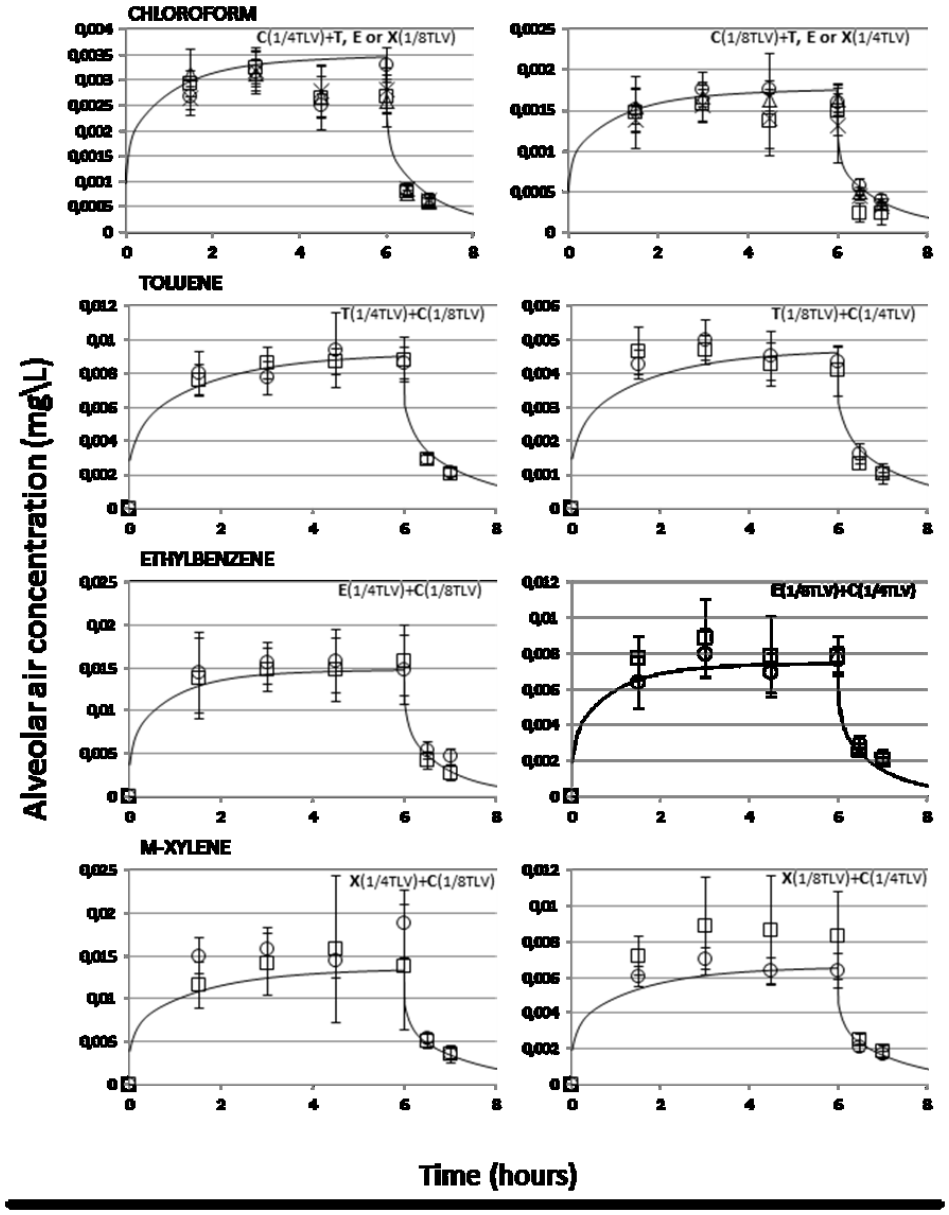

CHLOROFORM
C(1/4TLV)+T, E or X(1/8TLV)
C(1/8TLV)+T, E or X(1/4TLV)
TOLUENE
T(1/4TLV)+C(1/8TLV)
T(1/8TLV)+C(1/4TLV)
ETHYLBENZENE
E(1/4TLV)+C(1/8TLV)
E(1/8TLV)+C(1/4TLV)
M-XYLENE
X(1/4TLV)+C(1/8TLV)
X(1/8TLV)+C(1/4TLV)
Alveolar air concentration (mg\L)
Time (hours)

Figure 6

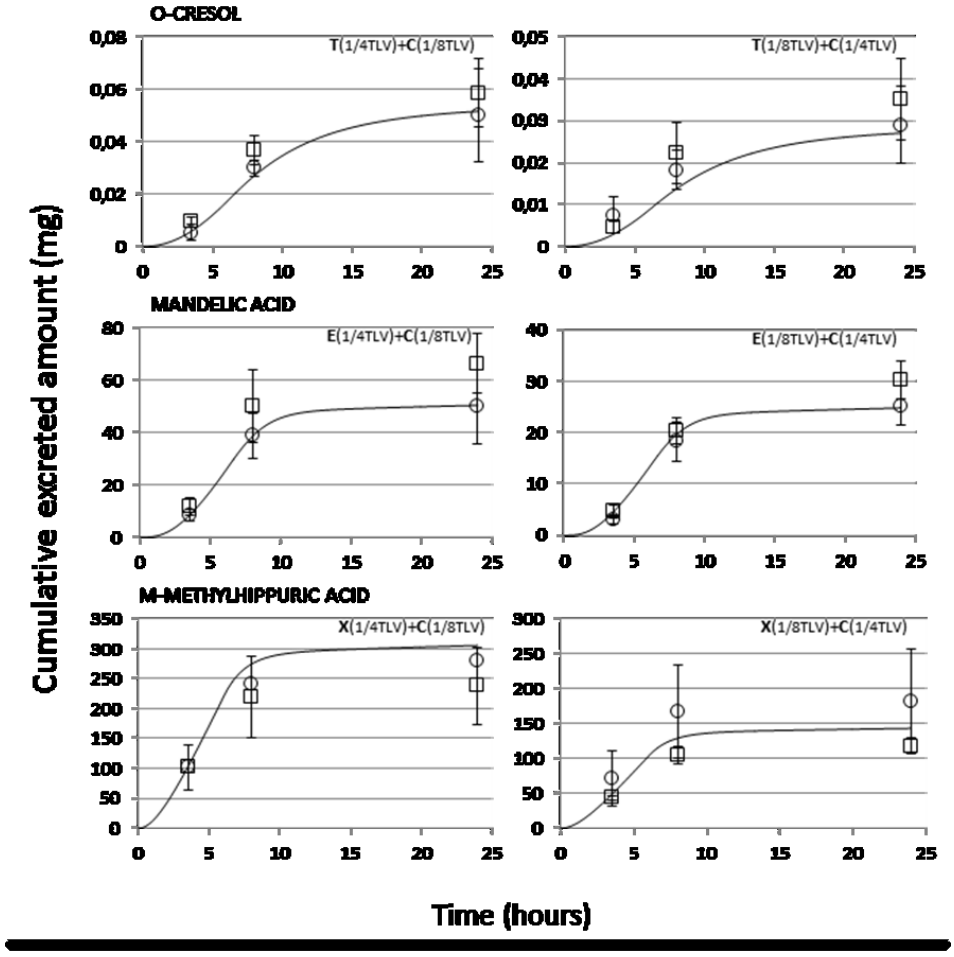

O-CRESOL
T(1/4TLV)+C(1/8TLV)
T(1/8TLV)+C(1/4TLV)
MANDELIC ACID
E(1/4TLV)+C(1/8TLV)
E(1/8TLV)+C(1/4TLV)
M-METHYLHIPPURIC ACID
X(1/4TLV)+C(1/8TLV)
X(1/8TLV)+C(1/4TLV)
Cumulative excreted amount (mg)
Time (hours)

Figure 7

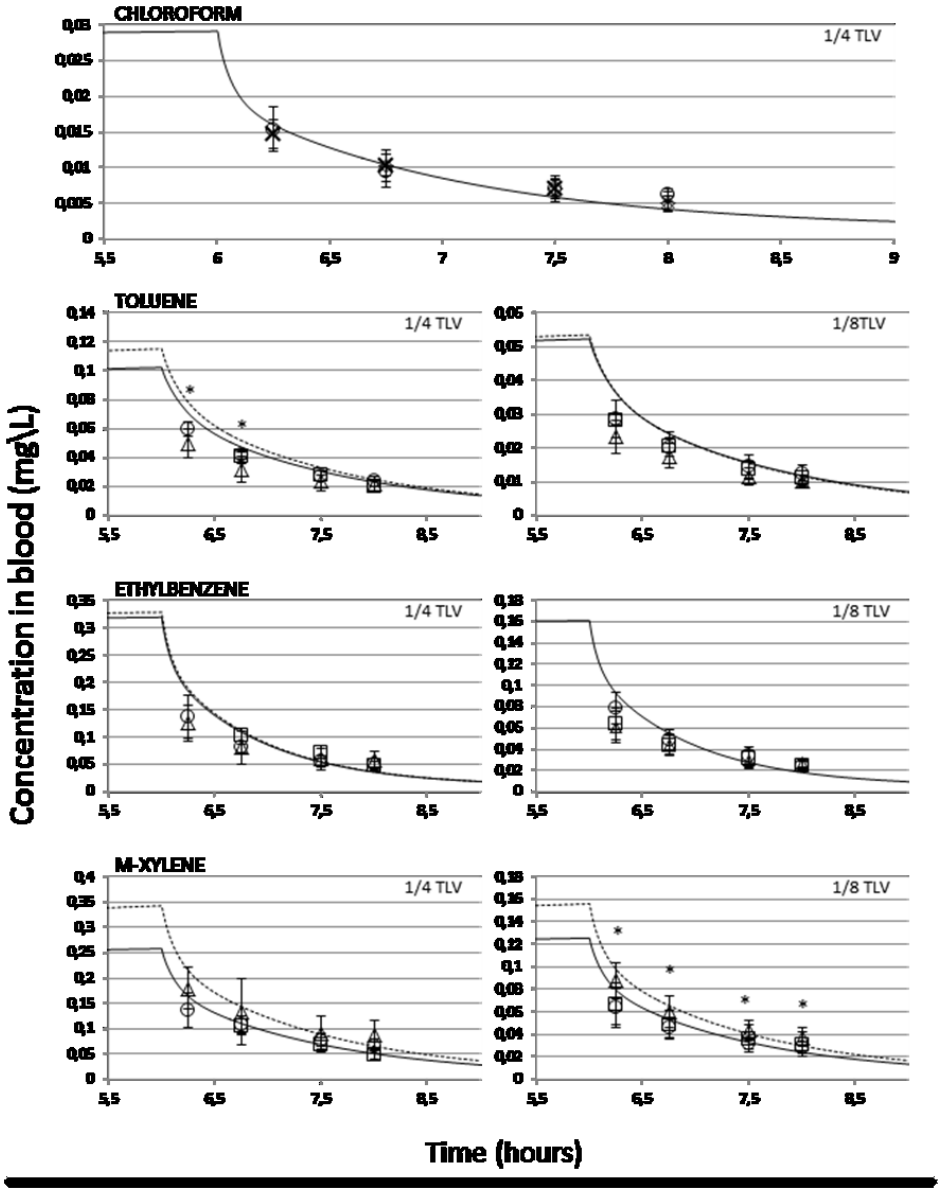

CHLOROFORM
1/4 TLV
TOLUENE
1/4 TLV
1/8TLV
ETHYLBENZENE
1/4 TLV
1/8 TLV
M-XYLENE
1/4 TLV
1/8 TLV
Concentration in blood (mg\L)
Time (hours)

Figure 8

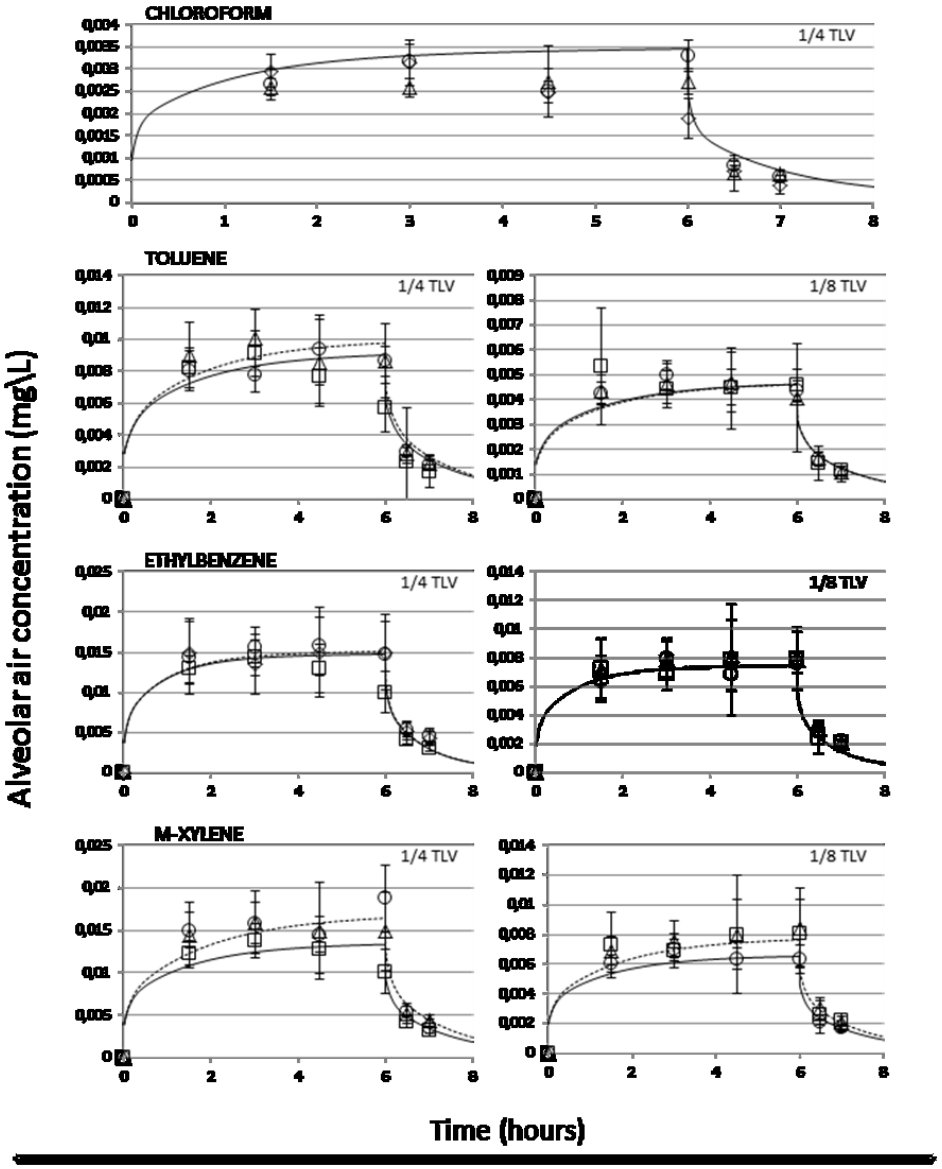

CHLOROFORM
1/4 TLV
TOLUENE
1/4 TLV
1/8 TLV
ETHYLBENZENE
1/4 TLV
1/8 TLV
M-XYLENE
1/4 TLV
1/8 TLV
Alveolar air concentration (mg\L)
Time (hours)

Figure 9

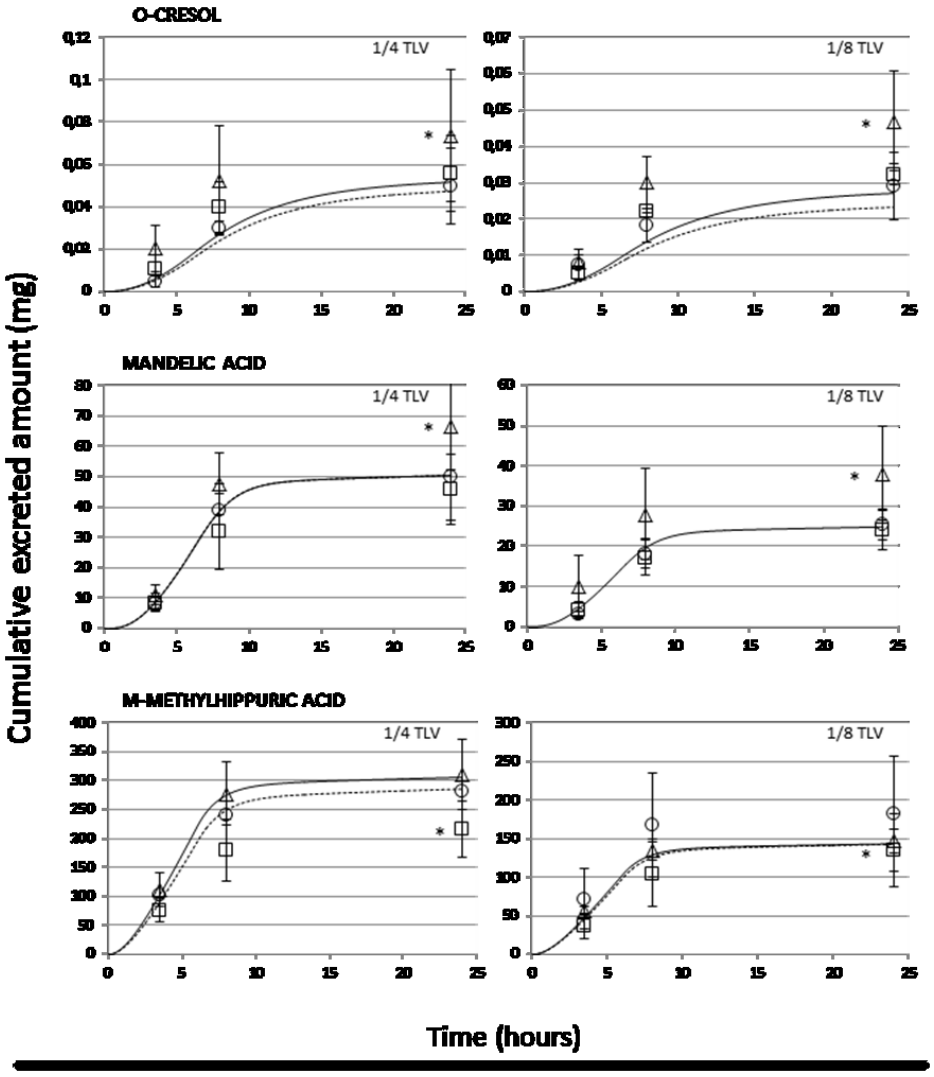
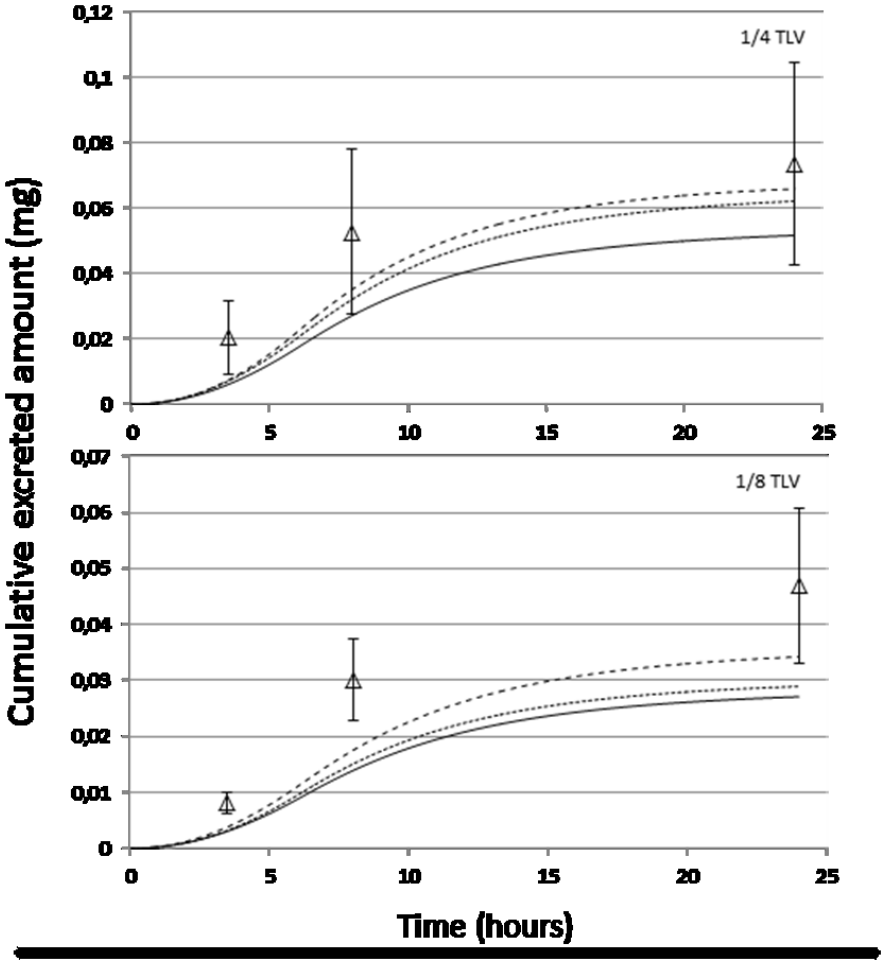

O-CRESOL
1/4 TLV
1/8 TLV
MANDELIC ACID
1/4 TLV
1/8 TLV
M-METHYLHIPPURIC ACID
1/4 TLV
1/8 TLV
Cumulative excreted amount (mg)
Time (hours)

Figure 10


1/4 TLV
1/8 TLV
Cumulative excreted amount (mg)
Time (hours)
0,12
0,1
0,08
0,06
0,04
0,02
0
0,07
0,05
0,03
0,01
5
10
15
20
25

3.11 Tables

Table 1. Exposure scenarios

VOC	Single exposures								Binary exposures						Quaternary exposures			
#	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18
C	1/4	1/8							1/4	1/8	1/4	1/8	1/4	1/8	1/4	1/4	<MAC	<MAC
T			1/4	1/8					1/8	1/4					1/4	1/8	1/4	1/8
E					1/4	1/8					1/8	1/4			1/4	1/8	1/4	1/8
X							1/4	1/8					1/8	1/4	1/4	1/8	1/4	1/8

MAC: Maximal admissible concentration for chloroform in drinkable water (90µg/L)

Table 2. Physiological parameter values for a standard adult human from Tardif et al. (1997)

Parameters	Symbol	Value
Body weight	BW	Average values measured for
Cardiac output	Qc	$18*BW^{0.7}$
Alveolar ventilation rate (L/hr/Kg)	Qalv	$18*BW^{0.7}$
Compartment volume (%BW)		
Adipose tissue	Vf	19
Liver	Vl	2.6
Richly perfused tissues	Vr	5
Poorly perfused tissues	Vmp	62
Blood flow to tissue compartment (%Qc)		
Adipose tissue	Qf	5
Liver	Ql	26
Richly perfused tissues	Qr	44
Poorly perfused tissues	Qmp	25

Table 3. Chemical specific parameters

Parameters	Symbol	CHL[a]	TOL[b]	EBZ[b]	m-XYL[b]
Partition Coefficients					
Blood :air		10.7	18	42.7	46
Liver:air		17	83.6	83.8	90.9
Adipose tissue :air		280	1021	1556	1859
Richly perfused tissues :air		17	83.6	60.3	90.9
Slowly perfused tissues:air		12	27.7	26	41.9
Metabolic constants					
Maximal rate (mg/hr/kg)	Vmax	12.68 3.4[e]	3.45[c]	13.32[d]	5.5 -
Affinity constant (mg/L)	Km	0.448	0.134	1.39	0.22

[a]From Haddad *et al,* 2006.
[b]From Tardif *et al,* 1997.
[c]From Haddad *et al*, 1999.
[d]From Marchand *et al*, 2014.
[e]This study.

Table 4. Urinary biomarkers excretion parameters

Biomarker	Parameters	Symbol	Value	Extrapolation
O-cresol	CYP2E1 model			
	o-CR excretion constant	K_{CRE}	0.715[a]	$BW^{-0.3}$
	Stoichiometric yield	SY	0.00059[b]	
	CYP1A2 model			
	o-CR first order constant (h^{-1}▪kg^{-1})	K_{TOL}	0.122[b]	$BW^{-0.3}$
Mandelic acid	2 step model			
	EBZ to MA (mg/hr/kg)	$Vmax_E$	13.32[b]	$BW^{0.75}$
	Stoichiometric yield	SY	0.9[c]	
	MA to PGA (hr^{-1})	K_{PGA}	0.265[b]	
	MA excretion constant (h^{-1})	K_{MA}	0.09[b]	
m-Methyl hippuric acid	MHA excretion constant	K_{MHA}	1.3[d]	
	Stoichiometric yield	SY_{MHA}	0.97[c]	

[a] Baelum *et al* (1985)
[b] Marchand *et al* (2014)
[c] Engström *et al* (1984)
[d] Riihimaki (1979)

Table 5. Metabolic inhibition constants for competitive inhibition for TEX from Tardif *et al* (1997)

Inhibitor	Substrate	Inhibition constant Ki
Toluene	Ethylbenzene	0.79
Toluene	m-Xylene	0.17
Ethylbenzene	Toluene	0.33
Ethylbenzene	m-Xylene	0.23
m-Xylene	Toluene	0.77
m-Xylene	Ethylbenzene	1.50

3.12 Appendix

Figure A-1

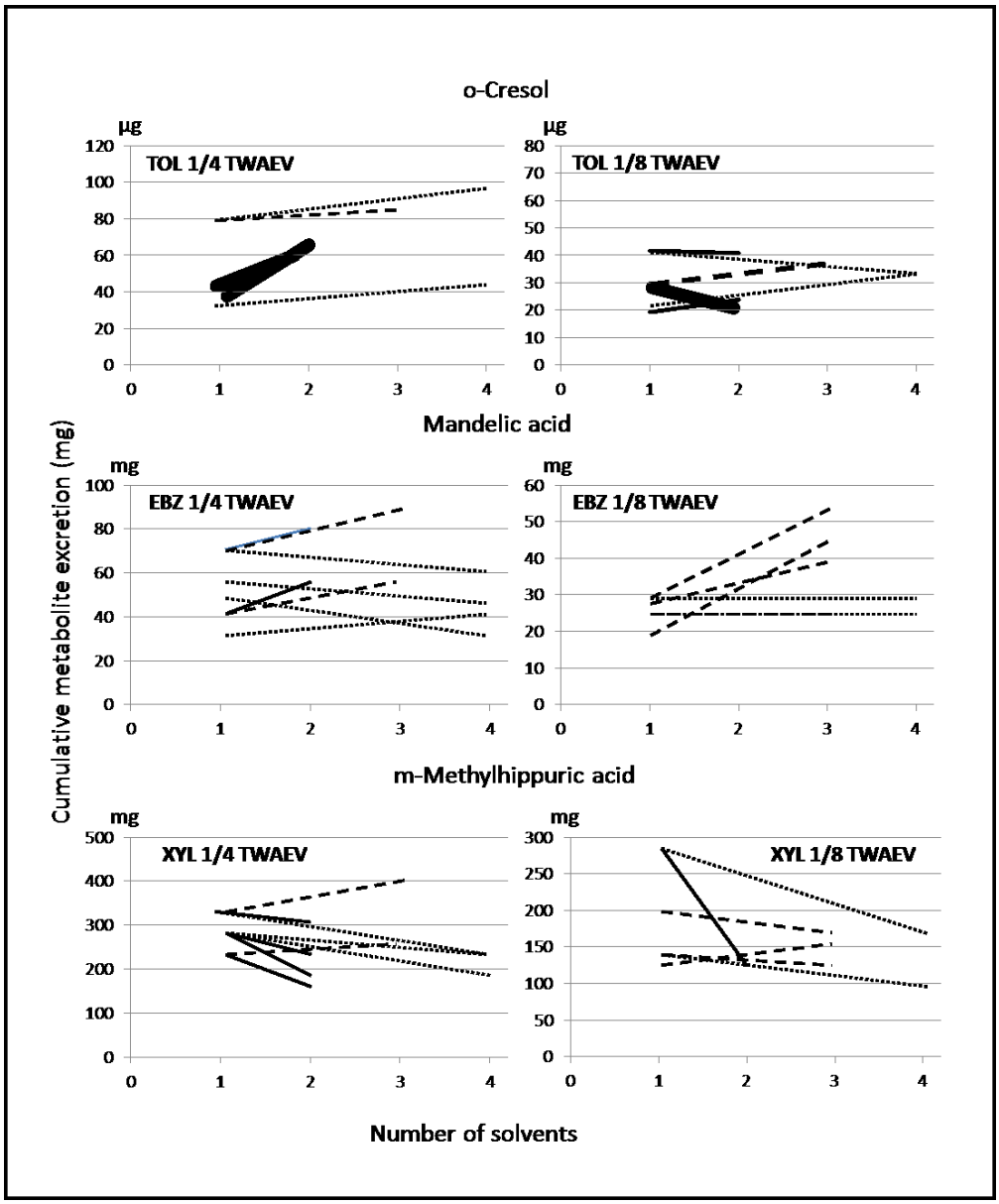


Figure A-2

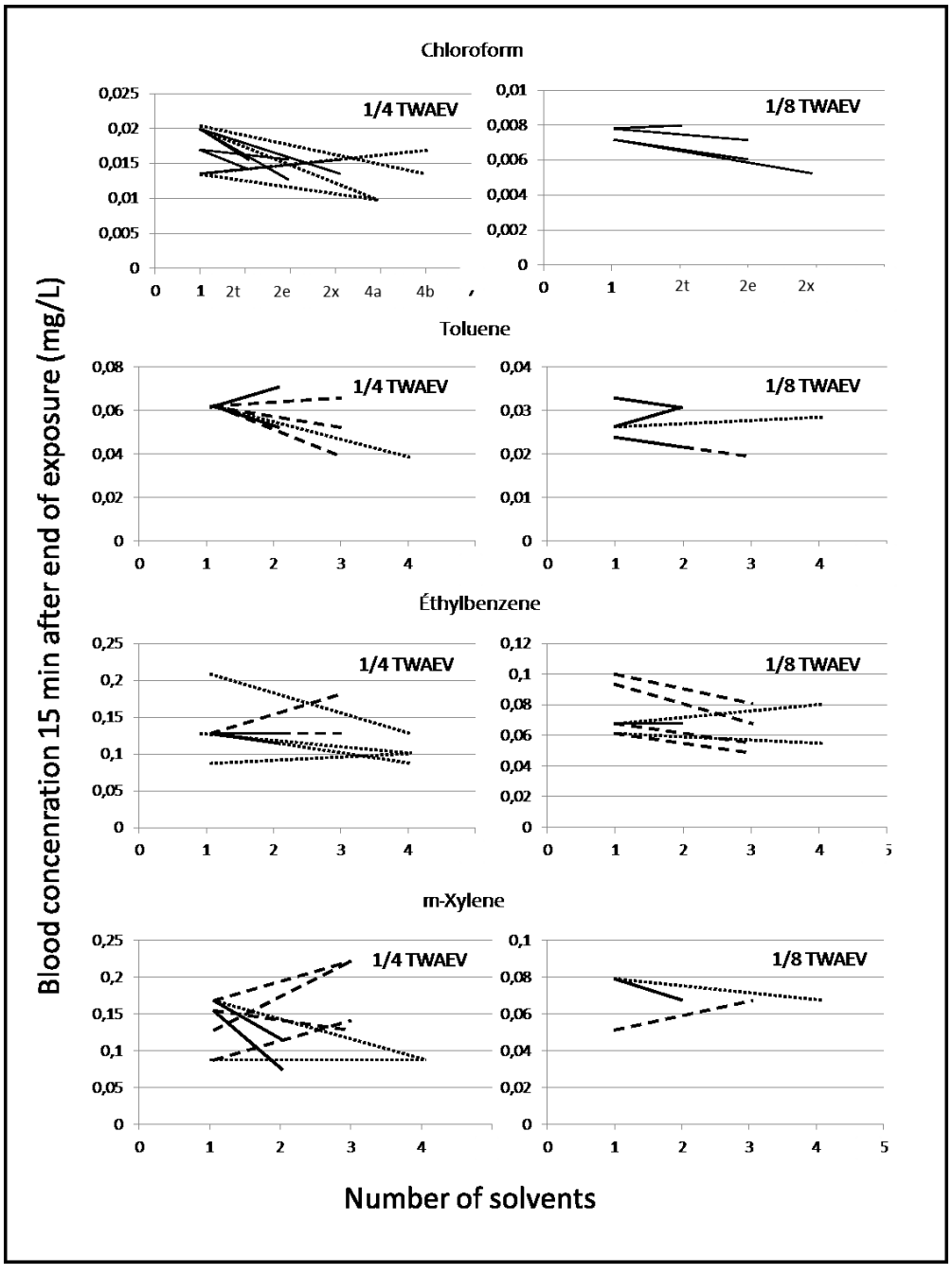

Chloroform
1/4 TWAEV
1/8 TWAEV
Toluene
1/4 TWAEV
1/8 TWAEV
Éthylbenzene
1/4 TWAEV
1/8 TWAEV
m-Xylene
1/4 TWAEV
1/8 TWAEV
Blood concenration 15 min after end of exposure (mg/L)
Number of solvents

Table A-1. Répartion des participants dans les différentes expositions.

Expo #	Solvents	Volonteers													
		A	B	C	D	E	F	G	H	I	J	K	L	M	N
1	C (1/4 TLV)	X	X	X	X	X									
2	C (1/8 TLV)	X		X	X	X									
3	T (1/4 TLV)	X		X	X	X	X								
4	T (1/8 TLV)	X		X	X	X	X								
5	E (1/4 TLV)	X	X							X		X	X		
6	E (1/8 TLV)							X				X	X	X	X
7	X (1/4 TLV)	X		X			X	X	X						
8	X (1/8 TLV)	X		X			X		X						
9	C (1/4 TLV) - T (1/8 TLV)	X		X			X		X	X					
10	C (1/8 TLV) - T (1/4 TLV)		X	X			X				X	X			
11	C (1/4 TLV) - E (1/8 TLV)	X		X			X	X		X					
12	C (1/8 TLV) - E (1/4 TLV)	X		X			X	X		X					
13	C (1/4 TLV) - X (1/8 TLV)	X	X							X	X	X			
14	C (1/8 TLV) - X (1/4 TLV)	X	X				X			X		X			
15	C (1/4 TLV), T (1/4 TLV), E (1/4 TLV), X (1/4 TLV)	X	X					X				X	X		
16	C (1/4 TLV), T (1/8 TLV), E (1/8 TLV), X (1/8 TLV)	X	X							X		X	X		
17	C (<MAC), T (1/4 TLV), E (1/4 TLV), X (1/4 TLV)	X					X	X		X					
18	C (<MAC), T (1/8 TLV), E (1/8 TLV), X (1/8 TLV)						X					X	X	X	X

Chapitre 4. Discussion générale et conclusion

Cette étude a permis de i) de récolter des données biologiques auprès de volontaires humain suite à des expositions simples, binaires ou quaternaires au chloroforme, au toluène, à l'éthylbenzène et au m-xylène ii) afin d'évaluer les potentielles interactions entre les différents composés organiques volatils et iii) de modéliser ses interactions en portant une attention particulière aux biomarqueurs urinaires.

D'abord, en ce qui a trait aux expositions aux substances seules, celles-ci ont permis de développer des modèles PBPK pouvant prédire les niveaux de biomarqueurs urinaires en fonction du niveau d'exposition et du temps. Les modèles existants pour l'o-cresol et l'acide m-methylhippurique n'ont pu permettre une bonne corrélation entre les métabolites urinaires et le niveau d'exposition principalement à cause des valeurs de Km et de Vmax utilisées. Afin de permettre une meilleur ajustement aux des données expérimentales observées pour les substances mères dans le sang, les clairances intrinsèques du toluène et de l'éthylbenzène se devaient d'être plus élevées que celles obtenues par Tardif *et al* (1997), alors que les données pour le chloroforme suggéraient une clairance plus faible que celle proposée par Corley *et al* (1990). Il est possible que ces différences au niveau des paramètres cinétiques soient dues à l'absence de données sanguines durant l'inhalation. Un changement de clairance entraine une plus grande différence pour les prédictions des niveaux sanguins vers la fin de la période d'inhalation que pour les prédictions post-expositions. Comme les données obtenues dans l'air expiré présentent une grande variabilité, elles ne peuvent compenser pour l'absence de données sanguines. Par exemple, le Vmax pour l'éthylbenzène optimisé par Tardif *et al* (1997) se base sur les données sanguines (7,3 mg/h/kg) alors qu'une optimisation en fonction des données pour l'air alvéolaire aurait donné un Vmax de 15,2 mg/h/kg si on maintient le Km à une valeur de 1,39 mg/L et un Vmax de 14,64 mg/h/kg si l'on optimise à la fois en fonction des valeurs sanguines et alvéolaires. Cette dernière valeur est très près de celle proposée dans l'article 1 (13,32 mg/h/kg). Également, Sams *et al* (2004) ont observé à l'aide de microsomes humains un Km de 0,85 mg/L et un Vmax de 8,5[1] mg/h/kg attribuable au CYP2E1, ce qui donne un ratio quasiment identique à celui optimisé dans la

[1] Valeur convertie selon Loizou et al, (1999)

présente étude. Dans une autre étude évaluant les niveaux sanguins à la suite d'une exposition à 25 ppm d'éthylbenzène, Knecht *et al* (2000) ont rapporté une valeur d'environ 0,3 mg/L après 4h d'inhalation. Si l'on regarde la Figure 4.1, on remarque que cette valeur est très près des prédictions du modèle optimisé dans la présente étude pour l'éthylbenzène comparativement au 0,38 mg/L prédit par le model de Tardif. Également, comme la vitesse du métabolisme varie en fonction de l'âge (Lazzer *et al*, 2010), il est possible que l'âge moyen relativement bas des participants (moins de 25 ans) ait pu contribuer à cet écart avec les études antérieures. L'effectif restreint de participants constitue également un important affectant la variabilité.

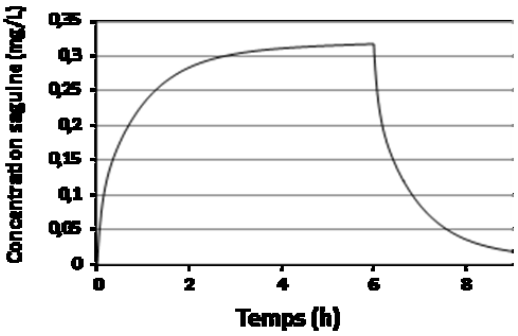


Figure 4.1 Simulation TCBP des niveaux sanguins en éthylbenzène suite à une exposition à 25 ppm avec un Vmax de 13,32 mg/h/kg.

Mis à part le CYP2E1, d'autres enzymes comme le CYP2B6 sont reconnues pour contribuer au métabolisme des composés à l'étude. Le CYP2E1 est reconnu comme étant l'enzyme principale à faibles concentrations de COV, alors que la contribution de CYP2B6 est plus grande à hautes concentrations d'exposition (Kim *et al,* 1997; Nakajima *et al,* 1997; Tassaneeyakul *et al,* 1996). La contribution de chaque composante enzymatique varie donc en fonction des concentrations en COV au foie. Après l'arrêt de l'exposition, les concentrations internes des COV diminuent. Il est possible que l'absence de données durant la période d'inhalation dans la présente étude entraine l'optimisation de paramètres

cinétiques se rapprochant davantage de l'activité du CYP2E1. Comme le CYP2B6 possède généralement un grand Km et une clairance intrinsèque moindre que le CYP2E1 (Tassaneeyakul *et al,* 1996), une augmentation des concentrations au foie pourrait tendre vers une cinétique se rapprochant de celle du CYP2B6. Si les valeurs de Km et Vmax pour le toluène et le chloroforme retenues dans la présente étude ne correspondent pas aux valeurs rapportées *in vitro* (Lipscomb *et al*, 2004), la clairance intrinsèque du toluène comparée à celle du chloroforme propre au CYP2E1 possède le même rapport que celui obtenu pour les clairances observées suite aux expositions simples au toluène et au chloroforme. Il aurait bien sûr été intéressant d'obtenir des données sanguines durant les périodes d'inhalation afin de vérifier la pertinence des changements apportés aux paramètres cinétiques.

En ce qui a trait aux différences statistiquement significatives observées pour les expositions mixtes comparativement aux expositions simples, l'attribution de la plupart de ces variations aux changements de participants dans l'étude semble plus probable que l'implication d'un quel qu'autre mécanisme. Par exemple, il est difficile d'expliquer les diminutions au niveau de l'excrétion urinaire de l'acide m-méthylhippurique, alors qu'aucun changement dans le sang n'est observé (expositions 15 et 16). Une altération au niveau de l'ADH ou de la conjugaison à la glycine pourrait entrainer de tels résultats, mais les concentrations retrouvées dans cette étude sont loin d'excéder la capacité maximale de conjugaison à la glycine d'environ 8.1 µmol/kg/min (Gregus *et al,* 1993) et une atteinte à l'activité enzymatique de l'ADH aurait contribué à une augmentation de l'excrétion de l'acide mandélique, ce qui n'a pas été observé pour le mélange CTEX. Cela vaut également pour l'augmentation de l'excrétion urinaire de l'acide mandélique observée pour les expositions à TEX (expositions 17 et 18). Si une altération de l'ADH était à l'origine de cette augmentation, une diminution des niveaux d'acide m-methylhippurique aurait été observée. Cependant, une étude réalisée par Chapman et al, (1990) révèle que la conversion de l'alcool benzoïque en acide benzoïque se ferait via le CYP450 plutôt que par l'ADH. Il est donc possible que la conversion de l'acide mandélique en acide phénylglyoxilique découle de l'activité de l'ADH alors que la formation d'acide benzoïque relève d'une autre enzyme et qu'à ce moment, les

contradictions décrites auparavant ne soient plus limitantes dans l'interprétation des résultats.

En ajustant les résultats urinaires pour le contenu en créatinine, on obtient des tendances semblables aux précédentes pour chacun des biomarqueurs urinaires entre les différentes expositions, mais les coefficients de variation sont généralement plus grands, ce qui contribue à rendre l'ensemble des résultats non significatifs dans l'urine (résultats non montrés). Il est donc plausible que la variabilité interindividuelle soit à l'origine d'au moins une part des résultats obtenus.

Dans l'ensemble, les modèles adaptés dans cette étude permettent de corréler les niveaux d'o-crésol et d'acides mandélique et m-méthylhippurique aux niveaux d'exposition au toluène, à l'éthylbenzène et au m-xylène et ce, que les substances soient seules ou en mélanges. La modélisation toxicocinétique des mélanges de COV suivant l'hypothèse d'une inhibition compétitive a démontré qu'à faible concentration l'effet d'une telle interaction a très peu d'influence sur les résultats attendus dans l'air exhalé, le sang ou l'urine. Le modèle ajusté pour le chloroforme permet lui aussi une bonne corrélation entre les concentrations sanguines et les concentrations d'exposition, mais les niveaux employés dans la présente étude n'ont pas permis de révéler une quelconque interaction avec les autres solvants étudiés.

Toutefois, la comparaison des résultats avec la littérature suggère que les modèles développés dans cette étude ne peuvent peut-être pas s'appliquer à de plus grandes concentrations d'exposition mais pourront être adaptées au besoin avec l'aide de données pertinentes additionnelles, bien entendu. Le nombre faible de données recueillies dans la présente étude constitue une limite qui contribue aux variations observées entre les études. Il apporte néanmoins des informations pertinentes et utiles pour l'estimation de l'exposition à un mélange de solvants.

Dans un contexte populationnel où les concentrations environnementales sont généralement très basses, ces modèles pourront fournir un outil intéressant afin d'évaluer l'exposition des populations dans le cadre d'études de grande envergure telles que l'Enquête Canadienne sur les Mesures de Santé. En effet, les modèles TCBP peuvent permettre par calcul inverse d'estimer l'exposition à partir des données de biosurveillance récoltées en plus de tenir compte des possibles interactions entre plusieurs solvants présents simultanément. La disponibilité de ce type de données, bien qu'elle représente un défi d'interprétation, est également une opportunité appréciable pour l'évaluation des risques à l'échelle de la population. Ces données procurent également une base pour les décisions normatives visant à protéger la santé du public.

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