

Université de Montréal

Études expérimentales sur la toxicocinétique du 1-hydroxypyrene

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Université de Montréal

Faculté de génie des procédés et de génie chimique

par

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Études expérimentales sur la toxicocinétique du 1-hydroxypyrene

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SOMMAIRE

Les hydrocarbures aromatiques polycycliques (HAP) sont des contaminants ubiquistes de notre environnement dont plusieurs sont classés cancérogènes probables chez l'humain. Parmi les membres les plus étudiés figurent le pyrène et le benzo(a)pyrène (BaP). La mesure urinaire du métabolite hydroxylé du pyrène, le 1-hydroxypyrene (1-OHP), a été proposée pour la surveillance biologique de l'exposition globale aux HAP. L'objectif général du projet de recherche était donc de compléter nos connaissances sur la toxicocinétique du 1-OHP et, accessoirement, sur celle des métabolites du BaP chez le rat.

Premièrement, la cinétique d'excrétion urinaire des métabolites du BaP (3-hydroxyBaP (3-OHBaP) et BaP-4,5-dihydrodiol (BaP-4,5-diol)) a été étudiée et comparée avec celle du 1-OHP obtenue dans une étude antérieure. Après injection intraveineuse de BaP, le profil temporel du 3-OHBaP et du BaP-4,5-diol et le pourcentage de la dose de BaP excrétée dans l'urine sous forme de ces métabolites étaient semblables à ceux déterminés après administration orale. Selon la dose, de 0,043 à 0,085% était excrété sous forme de 3-OHBaP et 0,02 à 0,05% sous forme de BaP-4,5-diol après injection intraveineuse et de 0,051 à 0,062% sous forme de 3-OHBaP et 0,006 à 0,026% sous forme de BaP-4,5-diol après administration orale. Une bonne relation dose-excrétion était également observée sur le large intervalle de doses administrées. Ces résultats sont assez semblables à ceux obtenus précédemment pour le 1-OHP. Après application cutanée de BaP, 0,018 à 0,055% selon la dose était excrété sous forme de 3-OHBaP et 0,0011 à 0,0026% sous forme de BaP-4,5-diol. Ces résultats plus faibles résultent du fait que l'absorption cutanée du BaP est conditionnée par son métabolisme au niveau de la peau et que cette dernière agit comme barrière protectrice comme dans le cas du pyrène. Par comparaison, après application cutanée de pyrène, la cinétique d'élimination du 1-OHP dans l'urine était semblable à celle observée après administrations intraveineuse et orale et ceci est probablement dû au fait que le passage transcutané du pyrène dépend surtout de sa diffusion passive transmembranaire.

Deuxièmement, la cinétique d'excrétion urinaire du 1-OHP et de métabolites du BaP a été déterminée chez des rats exposés aux composés parents ou aux métabolites par voie intraveineuse. Cette expérience a démontré que la biotransformation de phase I n'était pas l'étape limitante dans la cinétique d'excrétion urinaire des métabolites après administration des composés parents.

Troisièmement, l'excrétion urinaire et biliaire du 1-OHP a été comparée chez des rats exposés à différentes doses de pyrène par voies intraveineuse et orale. Il apparaissait que seule une faible proportion de 1-OHP était excrétée dans l'urine quelle que soit la voie d'exposition (en moyenne 2,7% de la dose intraveineuse et orale de pyrène), comme il avait été constaté antérieurement, mais qu'il en allait de même pour la bile (en moyenne 8,9% de la dose intraveineuse et orale de pyrène) suggérant la présence importante d'autres métabolites que le 1-OHP. Les profils d'excrétion urinaire et biliaire étaient également semblables pour chaque voie d'exposition. De plus, une bonne relation dose-excrétion était apparente sur le large intervalle de doses administrées ($R^2 = 0,981$ à $0,999$).

Quatrièmement, l'effet de l'injection intraveineuse de mélanges binaires et ternaires de pyrène, en présence de naphthalène ou de BaP sur la cinétique d'excrétion urinaire du 1-OHP a été examiné. La présence de naphthalène ou de faibles doses de BaP n'altérait ni la cinétique ni la quantité de 1-OHP excrétée. Ces données suggèrent l'absence d'interaction dans des conditions d'exposition aux doses faibles de HAP que l'on retrouve dans l'environnement.

Cinquièmement, la cinétique de distribution et d'élimination de la radioactivité totale de même que, du pyrène et du 1-OHP mesurés spécifiquement a été étudiée après injection intraveineuse de pyrène- ^{14}C . Le pyrène administré était rapidement distribué dans les divers tissus et, préférentiellement, dans les tissus adipeux, sous forme inchangée. Il était rapidement biotransformé et éliminé presque entièrement (82%) de l'organisme en 24 heures, sous forme métabolisée. Le 1-OHP représentait un faible pourcentage des métabolites totaux formés (15%). Cependant, il correspondait à tout moment à une fraction constante du ^{14}C total présent dans l'urine (4,2%) et les fèces (9,4%). Il reflétait donc l'élimination globale du pyrène de l'organisme. En conclusion, l'ensemble des données supportent l'utilité du 1-OHP dans l'urine comme bioindicateur de l'exposition aux HAP.

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LISTE DES ABRÉVIATIONS

BaP:	benzo(a)pyrène
BaP-4,5-diol:	benzo(a)pyrène-4,5-dihydrodiol
CLHP:	chromatographie liquide à haute performance
HAP:	hydrocarbures aromatiques polycycliques
3-OHBaP:	3-hydroxybenzo(a)pyrène
1-OHP:	1-hydroxypyrene
°C:	celsius
g:	gramme
kg:	kilogramme
µg:	microgramme
mg:	milligramme
ng:	nanogramme
mol:	moles
µmol:	micromoles
nmol:	nanomoles
pmol:	picomoles
L:	litre
m ³ :	mètre cube

À Jean-François,
à mes parents
et à la mémoire de Marcel

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CHAPITRE PREMIER

INTRODUCTION GÉNÉRALE

1.1 Les hydrocarbures aromatiques polycycliques (HAP)

1.1.1 Sources et exposition

Les hydrocarbures aromatiques polycycliques (HAP) sont des contaminants ubiquistes reconnus comme étant prioritaires au Canada (Armstrong et Newhook, 1992; Meek *et al.*, 1994). Ils représentent une famille de substances reliées structurellement qui contiennent au moins deux noyaux benzéniques fusionnés et qui ne sont formés que de carbone et d'hydrogène (Loening et Merritt, 1985; Loening *et al.*, 1990).

Les HAP se retrouvent sous forme de mélanges complexes dans l'environnement (Lesage *et al.*, 1987; Pathirana *et al.*, 1994). Ils proviennent principalement des processus de pyrolyse et en particulier de la combustion incomplète de la matière organique (Nikolaou *et al.*, 1984). Ils sont également des constituants naturels des huiles de coupe et de plusieurs autres produits pétrochimiques (Lindstedt et Sollenberg, 1982). Les émissions de HAP résultent surtout d'activités anthropiques (industries, milieu de travail, automobiles, avions, fumée de cigarette, foyers domestiques) mais certains événements naturels tels que les feux de forêts et volcans y contribuent également (Blumer, 1976; Nikolaou *et al.*, 1984). Les HAP sont donc très répandus dans l'environnement. Ils sont présents dans l'air urbain (Halsall *et al.*, 1994; Lee *et al.*, 1995; Pathirana *et al.*, 1994) et industriel (Allaire *et al.*, 1993; Petry *et al.*, 1994), dans les aliments (Kolar *et al.*, 1975; Lijinsky, 1991), dans l'eau de certains lacs (Borneff, 1977; Wild et Jones, 1995), dans les sols et sédiments marins (Hites et Gschwend, 1981; Odense *et al.*, 1985; Wild et Jones, 1995; Youngblood et Blumer, 1975). Le milieu industriel reste toutefois associé aux émissions ou à la présence des plus grandes quantités de

HAP, en particulier les cokeries et les usines où les matériaux carbonés tels que le coke, le brai de goudron de houille et les huiles d'asphalte sont produits ou utilisés, surtout à des températures élevées (Bjorseth *et al.*, 1981; Lindstedt et Sollenberg, 1982). Lindstedt et Sollenberg (1982) ont en effet classifié les différents milieux de travail où l'exposition aux HAP peut se produire selon les niveaux de BaP dans l'air ambiant. Les niveaux d'exposition les plus élevés ($>10 \mu\text{g}/\text{m}^3$ ou $>39,6 \text{ nmol}/\text{m}^3$)¹ ont été retrouvés dans les industries de production du coke et du gaz, les industries d'aluminium et les manufactures d'électrodes de carbone. Les travaux tels que l'épandage de goudron, de créosote et d'asphalte lors du pavage des routes ou de la réfection des toitures comptent également parmi les sources d'exposition les plus importantes à ces contaminants (Bjorseth *et al.*, 1981; Lindstedt et Sollenberg, 1982).

L'exposition aux HAP dans la population générale se fait surtout par la nourriture et la fumée de tabac, mais également par contact avec l'air extérieur (Jongeneelen, 1994; Lioy *et al.*, 1988; Van Rooij *et al.*, 1994b; Zhao *et al.*, 1992b). Ce sont toutefois les travailleurs exposés au goudron de houille et aux produits dérivés de celui-ci qui sont exposés aux concentrations les plus élevées en HAP (IARC, 1984). L'exposition en milieu de travail se fait surtout par inhalation (ACGIH, 1991; IARC, 1984), mais la contribution du contact cutané peut être importante ou même prédominante dans certains milieux tels que l'industrie de production du coke et de l'aluminium, l'industrie pétrochimique et les travaux impliquant la manipulation de la créosote (Boogaard and Van Sittert, 1994; Van Rooij *et al.*, 1993a,c; 1994a). Parmi les individus fortement exposés, on peut inclure les

¹Il a été choisi de rapporter les quantités de HAP en unités molaires. Lorsque la publication originale exprimait les doses en grammes, la conversion en moles est également présentée sauf lorsqu'il s'agit de mélanges de HAP.

patients psoriasiques se traitant avec des onguents ou shampoings à base de goudron de houille (Hansen *et al.*, 1993b; Van Schooten *et al.*, 1994). Ces travailleurs ou patients sont donc souvent des populations cibles pour l'étude des HAP étant donné que leur exposition se détache largement de l'exposition de base de la population en général.

1.1.2. Effets cancérigènes des HAP

Un certain nombre de HAP individuels ont été classés comme cancérigènes probables chez l'humain par le Centre international de recherche sur le cancer (IARC, 1987). Certaines études épidémiologiques ont en particulier démontré une incidence accrue de cancers du poumon chez les travailleurs d'usines de production du coke et du gaz (Doll *et al.*, 1972; Redmond *et al.*, 1976). Il existe également des études montrant une incidence accrue de cancers du poumon chez les travailleurs des industries de production d'aluminium et des fonderies de fer et d'acier (IARC, 1984; Armstrong *et al.*, 1994). Une preuve suffisante chez l'animal et limitée chez l'humain que la créosote est cancérigène a par ailleurs été mise en évidence (IARC, 1985).

1.2 Évaluation du risque, surveillance environnementale et surveillance biologique de l'exposition aux HAP

Étant donné le potentiel cancérigène de plusieurs membres de la famille des HAP, il importe donc de bien contrôler l'exposition à ces substances. Au départ, l'évaluation de l'exposition aux HAP reposait sur l'analyse des concentrations atmosphériques. Des méthodes non spécifiques telles que

la détermination des matières solubles dans le benzène (MSB) ont été utilisées (OSHA, 1976). Cette méthode ne permet toutefois pas de révéler l'identité des HAP; d'autres composés organiques présents dans l'atmosphère peuvent également contribuer à ces MSB. Des techniques chromatographiques permettant d'identifier et de quantifier les HAP individuels présents dans les mélanges complexes ont par la suite été développées (Hansen *et al.*, 1991; Quilliam and Sim, 1988; Roussel *et al.*, 1992; Wise *et al.*, 1993). Les méthodes d'analyse les plus sensibles et selectives incluent la chromatographie liquide à haute performance (CLHP) avec détection par fluorescence et la chromatographie capillaire en phase gazeuse avec détection par spectrométrie de masse (Law and Biscaya, 1994). NIOSH (1985) a par ailleurs recommandé une procédure standard pour l'analyse de 16 HAP individuels majeurs dans l'air ambiant après séparation par CLHP. Souvent, on a également recours à une détermination des concentrations atmosphériques de BaP pour estimer l'exposition aux HAP étant donné son potentiel cancérigène. Ces mesures environnementales permettent seulement d'estimer la dose inhalée. Par contre, comme mentionné précédemment, l'exposition aux HAP en milieu de travail peut également se produire par voie cutanée. Par ailleurs, étant donné la forte tendance des HAP à s'adsorber à la surface de particules, il est difficile de les récupérer avec certains solvants (Dufresne *et al.*, 1987). Ce phénomène peut également influencer leur biodisponibilité et leur cinétique dans les voies respiratoires (Gerde *et al.*, 1991; Pelfrene, 1976).

D'un autre côté, la surveillance biologique qui vise à estimer l'exposition interne de l'organisme, c'est-à-dire la dose effectivement absorbée et éventuellement la dose aux organes cibles, s'avère une méthode complémentaire efficace pour l'évaluation du risque encouru par l'exposition aux HAP. Dans le processus de développement et de validation d'un bioindicateur d'exposition aux HAP, il importe

toutefois que l'on ait une bonne compréhension de la toxicocinétique des produits mères et de leurs métabolites dans l'organisme (Heinzow et Mc Lean, 1994; Lauwerys et Bernard, 1985). En effet, de plus en plus d'études épidémiologiques, notamment des études transversales en milieu de travail, tendent à incorporer des mesures de bioindicateurs d'exposition dans une démarche d'analyse du risque (McMichael, 1994; Smith et Suk, 1994). Or, dans ce type d'études, les prélèvements de liquides biologiques (sang, urine) ne s'effectuent habituellement qu'à un moment précis de l'année, du mois ou de la journée. Il devient donc particulièrement important de posséder des connaissances approfondies sur le comportement toxicocinétique du ou des bioindicateurs retenus dans l'étude afin de réellement comprendre la signification des mesures et de leur apporter une juste interprétation.

1.3. Deux HAP modèles: le benzo(a)pyrène (BaP) et le pyrène

Plus de 500 HAP ont été détectés dans l'air et plus de 300 HAP ont été identifiés dans le goudron de houille (Bjorseth, 1983). De ce nombre, 16 HAP, qui représentent souvent plus de 80 à 90% de la somme des HAP des mélanges, ont été classés prioritaires par le U.S. Environmental Protection Agency (U.S.E.P.A., 1979) (Figure 1). Parmi eux, on retrouve le pyrène et le benzo(a)pyrène (BaP).

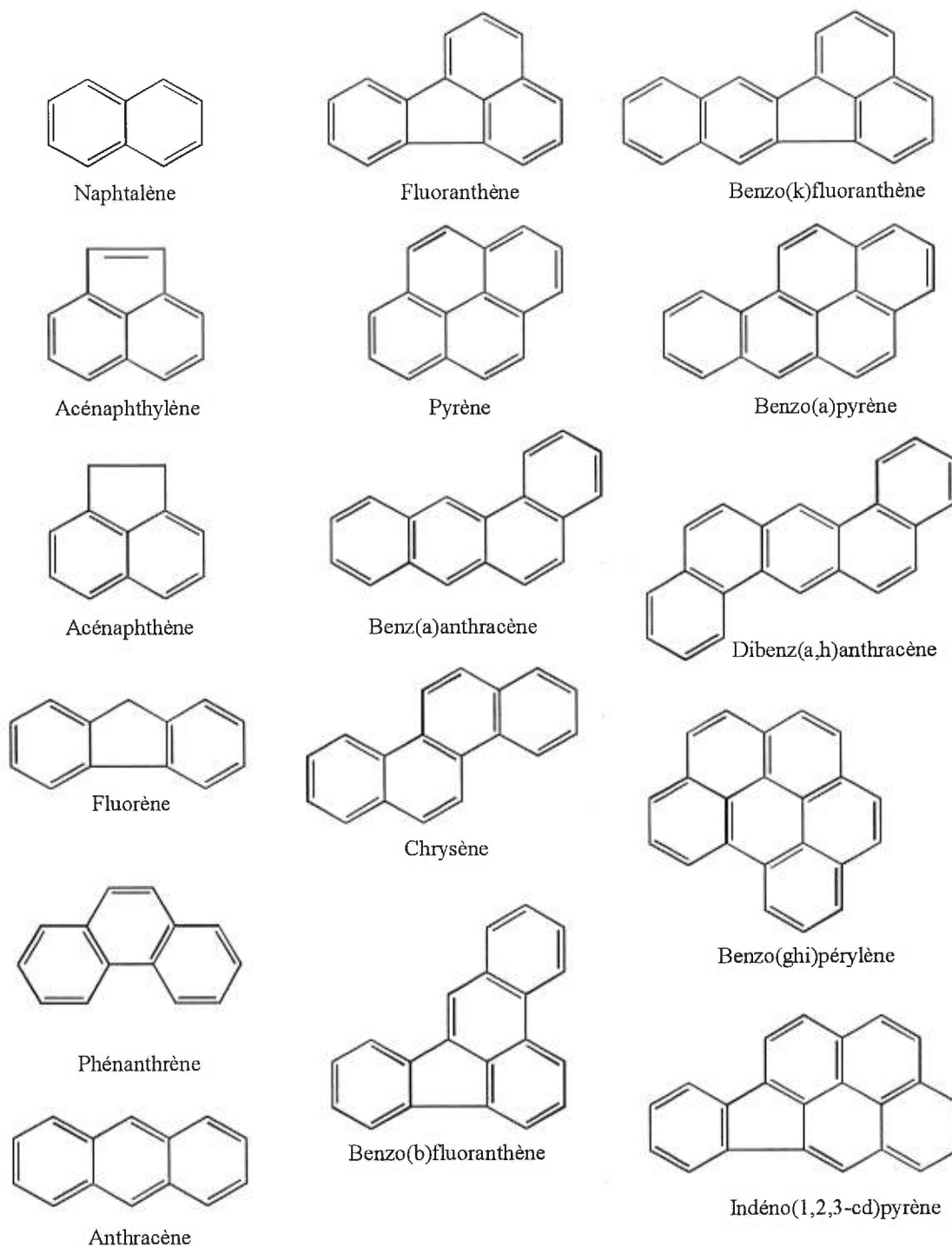


Figure 1. Les 16 HAP reconnus prioritaires par le U.S.E.P.A. (1979)

1.3.1. Le BaP

Le BaP est le HAP le mieux connu et le plus mesuré. Il figure parmi les HAP les plus communs dans l'environnement (NIOSH, 1985; U.S.E.P.A., 1979). On se réfère donc souvent à une mesure environnementale du BaP pour exprimer l'exposition globale aux HAP (Allaire *et al.*, 1993; Lindstedt et Sollenberg, 1982). Les HAP présents dans l'atmosphère sont distribués entre les phases gazeuse et particulaire (Lesage *et al.*, 1987; Nikolaou *et al.*, 1984). La distribution entre ces deux phases dépend principalement de la pression de vapeur de la phase liquide des HAP à la température de l'air ambiant. Des composés avec une pression de vapeur supérieure à 10^{-6} Torr à la température ambiante vont donc exister au moins partiellement dans la phase gazeuse dans l'atmosphère. Les HAP de plus faibles poids moléculaires, c'est-à-dire les HAP possédant de deux à quatre noyaux benzéniques, sont largement confinés à la phase gazeuse alors que les HAP de poids moléculaires plus élevés sont retrouvés en majeure partie adsorbés à des particules aéroportées (Atkinson et Arey, 1994). Étant donné sa faible pression de vapeur, le BaP se retrouve habituellement lié en majeure partie à des matières particulaires dans l'air urbain, industriel et dans des émissions dues à des combustions naturelles et à la fumée de cigarette (Dufresne *et al.*, 1987; Lee *et al.*, 1995; Lesage *et al.*, 1987)

Lorsque l'on pense aux effets sur la santé découlant de l'exposition aux HAP, on pense bien sûr surtout au BaP et à son potentiel cancérigène. En fait, le BaP a été classé cancérigène probable chez l'humain par le Centre international de recherche sur le cancer (CIRC) (groupe 2A) et par le U.S. Environmental Protection Agency (groupe B2) (IARC, 1987; U.S. Department of Health and Human Services, 1994); il est en outre un des membres de la famille des HAP ayant le potentiel cancérigène

le plus élevé (Collins *et al.*, 1991; Krewski *et al.*, 1989). Le BaP est un procancérogène qui requiert une activation enzymatique, principalement au niveau du foie, par les mono-oxygénases à fonctions multiples dépendantes du cytochrome P-450 pour exercer son effet cancérogène (Conney, 1982; Conney *et al.*, 1994). Il est métabolisé en une vingtaine de métabolites primaires et secondaires oxydés et en une variété de conjugués (Gelboin, 1980; IARC, 1983) (Figure 2). Plusieurs métabolites peuvent induire des mutations, transformations cellulaires et/ou se lier à des macromolécules cellulaires (Conney, 1982). Bien que plusieurs dihydrodiols, quinones et phénols aient été identifiés, seul le (+)-7R,8S-dihydroxy-9S,10R-époxy-7,8,9,10-tétrahydroBaP (encore appelé le (+)-7 β ,8 α -dihydroxy-9 α ,10 α -époxy-7,8,9,10-tétrahydroBaP, (+)-BaPDE-I ou trans-7,8-dihydroxy-9,10-époxy-7,8,9,10-tétrahydro-BaP) est retenu comme métabolite cancérogène ultime pouvant se lier de façon covalente à l'ADN (Conney, 1982; Conney *et al.*, 1994) (Figure 3).

1.3.2. Le pyrène

Le pyrène est retrouvé en importante proportion (souvent de 1 à 10%) dans la plupart des mélanges de HAP (Buchet *et al.*, 1992; Halsall *et al.*, 1994; Petry *et al.*, 1994; Roussel *et al.*, 1992). Il se retrouve partagé entre la phase gazeuse et la phase particulaire dans l'atmosphère (Atkinson et Arey, 1994).

Contrairement au BaP, le pyrène n'exhibe pas de potentiel cancérogène ni mutagène dans plusieurs systèmes biologiques (IARC, 1983). Le pyrène subit l'action des cytochromes P-450 pour former des phénols, dihydrodiols et diphénols qui peuvent être conjugués à du glucuronate, à du sulfate et

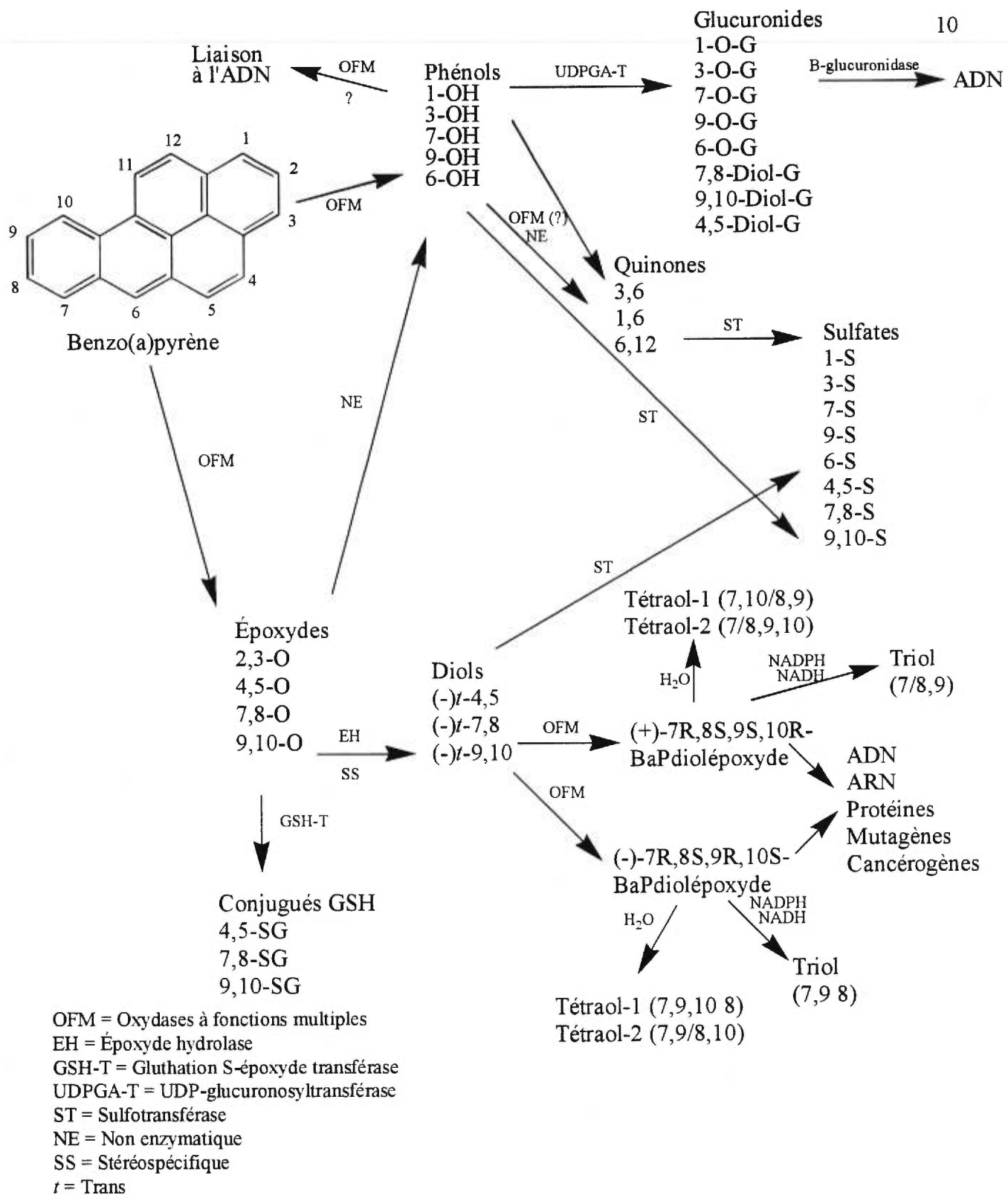


Figure 2. Métabolisme et activation du benzo(a)pyrène. (Adapté de Gelboin, 1980)

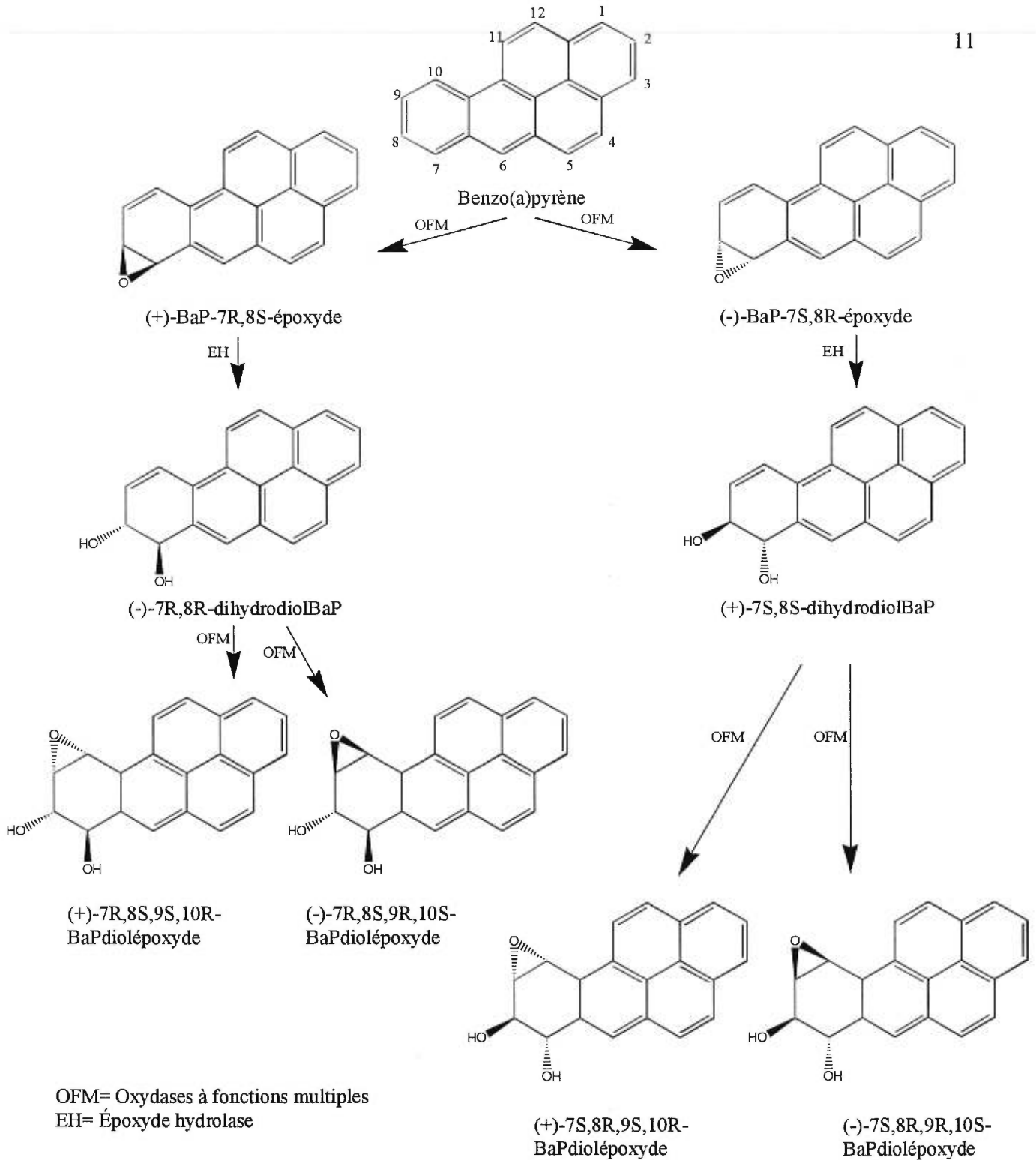


Figure 3. Métabolisme du BaP: Formation des diolépoxydes.
(Adapté de Conney, 1982)

au glutathion pour donner des métabolites secondaires hydrosolubles (Boyland et Sims, 1964) (Figure 4). Étant donné la symétrie de la molécule de pyrène, seuls deux sites d'oxydation primaires existent (Jacob *et al.*, 1982). Le 1-hydroxypyrene (1-OHP) est le métabolite majeur du pyrène produit par des microsomes de foie de rats *in vitro* (Jacob *et al.*, 1982) et le métabolite majeur dans l'urine de rat, de cochon et de lapin (Boyland et Sims, 1964; Keimig *et al.*, 1983).

1.4. Estimation de l'exposition par la mesure des métabolites urinaires du BaP et du 1-hydroxypyrene (1-OHP)

Dans une approche de surveillance biologique, la mesure des métabolites urinaires est couramment utilisée pour l'évaluation de la dose absorbée de produits chimiques. Il est à noter que la voie fécale semble en général la voie d'excrétion majeure des HAP ainsi que de leurs métabolites (Jacob *et al.*, 1989; Moody *et al.*, 1995; Van de Wiel *et al.*, 1993; Withey *et al.*, 1993b). Il reste tout de même que, dans une perspective de surveillance biologique de l'exposition chez l'humain, le recours à une mesure de métabolites excrétés dans les fèces demeure une approche difficilement applicable et donc difficilement réalisable en routine contrairement à une mesure urinaire.

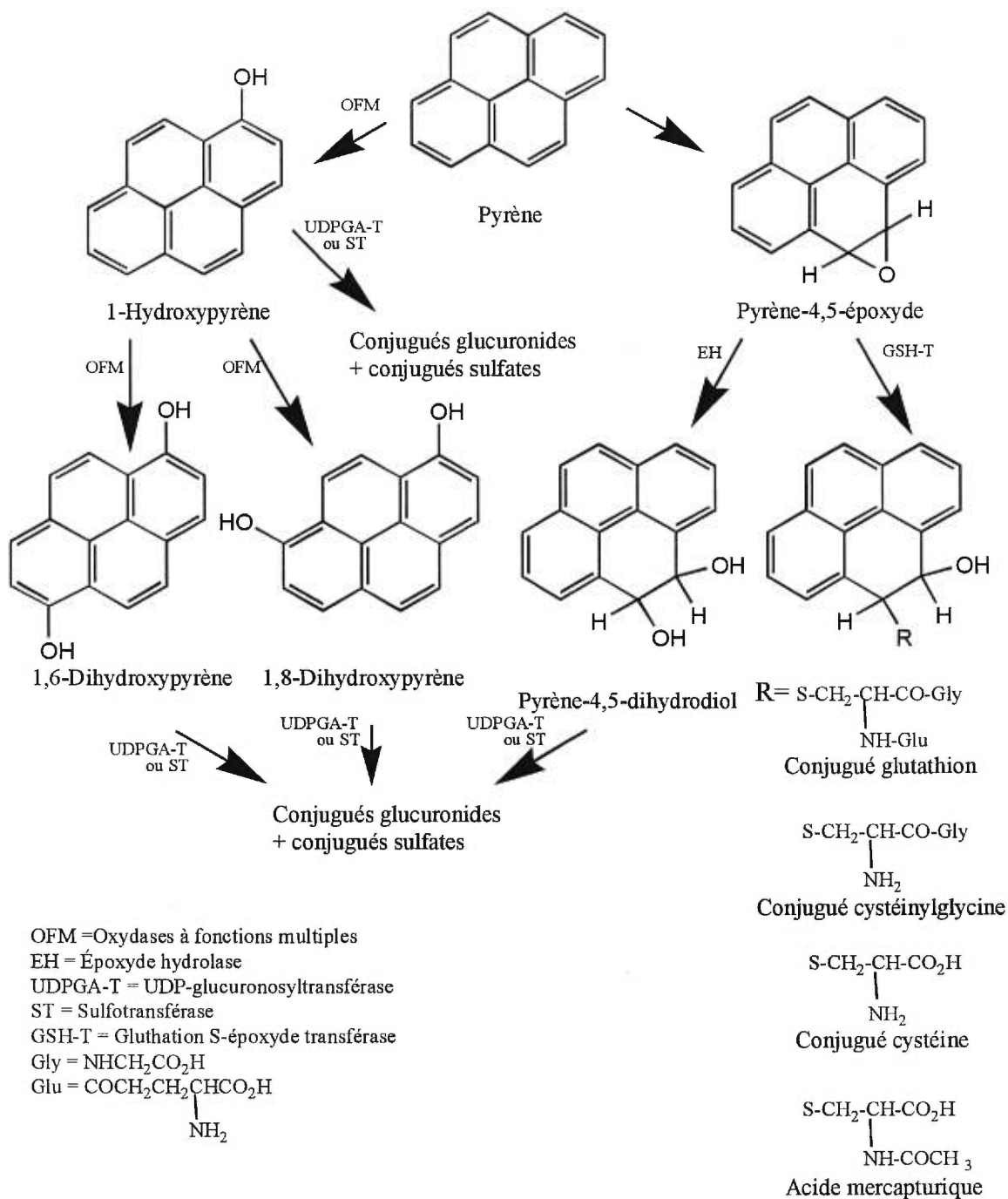


Figure 4. Métabolisme du pyrène. (Adapté de Boyland et Sims, 1964)

1.4.1 Métabolites urinaires du BaP

1.4.1.1. Importance et utilité

Étant donné le potentiel cancérigène du BaP et étant donné qu'il se retrouve comme composant de la plupart des mélanges de HAP, il convient dès lors de se demander s'il ne serait pas avantageux de recourir à la mesure de métabolites du BaP dans l'urine pour la surveillance biologique de l'exposition globale aux HAP. Notre propre expérience en ce domaine, conjuguée à un examen de la littérature, révèle que bien que le 3-hydroxyBaP (3-OHBaP) soit un métabolite majeur du BaP dans l'urine de souris et de rats, celui-ci n'est pas mesurable chez les travailleurs exposés aux produits du goudron de houille (Jongeneelen *et al.*, 1986) ou dans la population générale (Jongeneelen *et al.*, 1986) avec les méthodes d'analyse courantes par CLHP ou par chromatographie en phase gazeuse (GC). Par ailleurs, seules de très faibles concentrations ont été mesurées chez des patients traités avec des onguents à base de goudron de houille les exposant à de très fortes doses de BaP (Jongeneelen *et al.*, 1986).

Il faut donc avoir recours à des méthodes extrêmement sensibles pour quantifier ces métabolites du BaP dans l'urine humaine. Selon la méthode de Jongeneelen *et al.* (1987), utilisée couramment pour le dosage du 1-OHP urinaire et basée sur une hydrolyse enzymatique des formes glucurono- et sulfo-conjuguées, extraction en phase solide et détection par CLHP/fluorescence, le 3-OHBaP n'était pas détecté dans l'urine de travailleurs exposés aux HAP. La limite de détection rapportée était de 100 ng/L (373 pmol/L) approximativement. Cette méthode reste toutefois très sensible pour le dosage du 1-OHP chez l'animal et l'humain, y compris chez les individus sans exposition en milieu de travail ou

pour le dosage des métabolites du BaP chez l'animal.

Parmi les métabolites majeurs du BaP mesurés spécifiquement dans l'urine de rats et de souris, on compte principalement le 3-OHBaP, le 7,8-dihydrodiolBaP (7,8-diolBaP) et le 4,5-dihydrodiolBaP (4,5-diolBaP) mais également le 9,10-dihydrodiolBaP (9,10-diolBaP) et le 9-hydroxyBaP (9-OHBaP) en plus faibles proportions (Camus *et al.*, 1984; Jongeneelen *et al.*, 1984; Likhachev *et al.*, 1992; Tyndyk *et al.*, 1994; Van de Wiel *et al.*, 1993).

Les différences entre animal et humain s'expliquent entre autres par une activité hydroxylante du BaP plus faible dans des foies humains que dans ceux de rongeurs (Selkirk *et al.*, 1983). Des études *in vitro* utilisant des cytochromes P450 humains ont montré que l'isoenzyme P450 1A1 est le plus actif des P450s humains à former tous les produits d'oxydation du BaP à l'exception du 4,5-diolBaP et de certains matériaux polaires éluant avec le front de solvant lors de l'analyse par CLHP (Bauer *et al.*, 1995). Le P450 1A1 humain catalyse principalement la formation du 3-OHBaP, un produit de détoxification, et du 7,8-diolBaP, un produit d'activation, soit un cancérigène proximal (Bauer *et al.*, 1995). Par contre, le niveau d'expression du P450 1A1 est faible dans la majorité des foies humains adultes. Jongeneelen *et al.* (1986) montra d'ailleurs que l'excrétion urinaire du 3-OHBaP après application cutanée de goudron de houille chez le rat était beaucoup plus élevée que celle de patients psoriasiques exposés à une dose comparable. Jongeneelen (1987) a également déterminé, par des études *in vitro* utilisant des préparations de surnageant de foies humains après centrifugation à 9000 g, les constantes cinétiques (K_m et V_{max}) des isoenzymes P450s impliquées dans l'hydroxylation du pyrène et du BaP. Ils confirmèrent que la valeur de V_{max} moyenne pour l'hydroxylation du pyrène

était dix fois plus élevée que celle pour l'hydroxylation du BaP. La valeur de K_m moyenne des isoenzymes envers le BaP et le pyrène était du même ordre de grandeur.

Chez l'humain, les concentrations de métabolites hydroxylés majeurs du BaP dans l'urine, notamment le 3-OHBaP, sont de l'ordre du ng/L (pmol/L) (Ariese *et al.*, 1994; Grimmer *et al.*, 1991). En ayant recours à une méthode très sensible de chromatographie en phase gazeuse avec détection par spectrométrie de masse permettant la détection de concentrations de l'ordre du ng/L (pmol/L), Grimmer *et al.* (1991) identifièrent des métabolites du BaP chez des travailleurs d'une usine de coke, chez des travailleurs affectés au pavage des routes et chez des individus non exposés. Ils rapportèrent, respectivement, des valeurs 60 et trois fois plus élevées de métabolites totaux du BaP chez des travailleurs d'une usine de coke et chez des travailleurs de la route par rapport à des contrôles. Ces auteurs identifièrent un mélange de 3-OHBaP et 9-OHBaP comme pic majeur de la série des dérivés hydroxy des HAP avec une masse moléculaire de 252.

Ariese *et al.* (1994) ont également quantifié du 3-OHBaP chez des travailleurs de fours à coke et chez des contrôles non exposés. À cette fin, une méthode très sensible, soit la chromatographie liquide à haute performance avec détection par spectrométrie avec excitation laser Shpol'skii, leur a permis de détecter des concentrations aussi faibles que 0,5 ng/L (1,9 pmol/L). La concentration moyenne était de 8,3 ng/L (30,9 pmol/L) chez les contrôles. Likhachev *et al.* (1992) mentionnèrent, par ailleurs, avoir détecté du 3-OHBaP et 7,8-diolBaP dans l'urine de patients atteints du cancer du poumon qui étaient de grands fumeurs.

1.4.1.2. Cinétique de l'excrétion urinaire des métabolites du BaP chez l'animal

Plusieurs chercheurs se sont intéressés à la mesure urinaire de métabolites du BaP chez l'animal, la plupart du temps suite à des expositions aiguës. Ils cherchaient surtout à identifier les divers métabolites majeurs du BaP dans l'urine (Camus *et al.*, 1984; Van de Wiel *et al.*, 1993), à examiner l'excrétion urinaire en rapport avec l'excrétion fécale (Likhachev *et al.*, 1992; Van de Wiel *et al.*, 1993), à établir l'influence de la voie d'administration sur la quantité de métabolites spécifiques excrétés dans l'urine (Jongeneelen *et al.*, 1984; Van de Wiel *et al.*, 1993) ou encore à identifier des bioindicateurs de susceptibilité à l'effet cancérigène du BaP (Likhachev *et al.*, 1992). À cette fin, des collectes de 24 heures (Likhachev *et al.*, 1992; Van de Wiel *et al.*, 1993) ou de 72 heures (Camus *et al.*, 1984) ont été effectuées. De ces données, seuls des profils cinétiques très grossiers de l'excrétion des métabolites du BaP dans l'urine ont pu être déterminés.

Entre autres, Likhachev *et al.* (1992) exposèrent des rats LIO à une dose intrapéritonéale aiguë de 200 mg de BaP/kg (793 $\mu\text{mol/kg}$) et analysèrent le 3-OHBaP et le 7,8-diolBaP dans des collectes urinaires de 24 heures effectuées sur une période de cinq à 30 jours. Ces auteurs observèrent que le 7,8-diolBaP était excrété en quantité plus importante que le 3-OHBaP. L'excrétion maximale de ces métabolites était atteinte aux jours deux et trois suivie par une élimination lente. Les deux métabolites étaient encore quantifiables deux semaines après l'exposition mais l'élimination était complète à l'intérieur de 30 jours. Tyndyk *et al.* (1994) exposèrent des rats LIO mâles à dix doses de 10 mg de BaP/kg (39,6 $\mu\text{mol/kg}$) administrées à intervalles de dix jours. Des collectes urinaires de 24 heures ont été effectuées sur une période de huit jours et ce après la première, la cinquième et la dixième

injection. Suite à la première injection, ces auteurs observèrent une cinétique d'excrétion particulière ne permettant pas de déterminer de demi-vie apparente d'élimination. En effet, les métabolites étaient quantifiables pendant les huit jours suivant l'exposition mais aucun pic d'excrétion n'était apparent à un jour particulier et aucun patron d'élimination progressive dans le temps n'était observé. Ces auteurs rapportèrent également une diminution de l'excrétion du 3-OHBaP et du 7,8-diolBaP dans des collectes effectuées après la cinquième et la dixième injection par rapport à celles qui étaient effectuées après une injection. Ils ont associé cette diminution à un changement dans la capacité des enzymes à métaboliser le BaP avec l'âge.

Par ailleurs, Van de Wiel *et al.* (1993) étudièrent l'excrétion urinaire et fécale du BaP et de ses métabolites majeurs chez des rats Wistar mâles et femelles. Des collectes urinaires de 24 heures ont été effectuées durant les quatre jours suivant l'administration de 1 mmol de BaP/kg par voies orale et intrapéritonéale ainsi que le septième jour après le traitement. Le 3-OHBaP était le métabolite prédominant dans l'urine. Le 9-OHBaP, 9,10-diolBaP et 4,5-diolBaP étaient excrétés en quantités 100 fois moindres. Les quantités de 7,8-diolBaP excrétées étaient 1000 fois plus faibles que celles du 3-OHBaP. Ces auteurs ont également rapporté qu'après administration orale, l'excrétion des métabolites totaux était maximale aux jours un et deux après exposition tandis que le pic d'excrétion était observé aux jours deux et trois suivant l'injection intrapéritonéale. Enfin, Jongeneelen *et al.* (1986) ont également étudié l'excrétion urinaire du 3-OHBaP après application cutanée de 53 mg de goudron de houille contenant 9,5 mg de BaP/g (37,7 $\mu\text{mol/g}$) chez des rats Wistar mâles. Ils ont montré que l'excrétion du 3-OHBaP était maximale au jour deux suivant l'exposition et que 96% du BaP excrété dans l'urine pendant la période de collectes de cinq jours (collectes consécutives de 24

heures) étaient éliminés dans les premières 72 heures. Cependant, comme dans le cas des études de Likhachev *et al.* (1992), Tyndyk *et al.* (1994) et Van de Wiel *et al.* (1993), le protocole utilisé ne permettait de tracer qu'une cinétique grossière de l'excrétion des métabolites.

Dans une récente publication, nous avons toutefois déterminé le profil détaillé d'excrétion urinaire du 3-OHBaP chez des rats mâles Sprague-Dawley suivant une injection intrapéritonéale unique de 31,25 μmol de BaP/kg (Bouchard et Viau, 1995). Les urines furent recueillies à intervalles fréquents sur une période de 48 ou 164 heures. Un plateau persistant sur une période d'un moins 48 heures suivant l'administration était initialement atteint. La cinétique montrait ensuite au moins deux phases d'élimination et des demi-vies apparentes de premier ordre de 6,7 et 25 heures ont été calculées pour les périodes 52 à 72 heures et 72 à 164 heures après le traitement, respectivement. Par ailleurs, 94% de la quantité totale de 3-OHBaP excrétée pendant la période de collectes de 164 heures ont été éliminés dans les premières 72 heures après administration.

Il est également intéressant de noter que les données sur la cinétique d'excrétion urinaire de métabolites du BaP suivant une exposition répétée sont limitées. Outre l'expérience de Tyndyk *et al.* (1994) mentionnée précédemment, on compte celles de Jongeneelen *et al.* (1984; 1985b) qui ont exposé des rats Wistar mâles à 10, 20 et 50 μmol de BaP/kg par jour pendant trois jours consécutifs par voies orale et cutanée. Ils ont effectué des collectes de 24 heures sur une période de six jours suivant le début des administrations et ont rapporté que l'exposition répétée au BaP résultait en une augmentation de l'excrétion urinaire du 3-OHBaP. Suivant la fin du traitement, l'élimination était rapide et presque complète quatre jours après le dernier dosage. Récemment, nous avons déterminé

le profil cinétique du 3-OHBaP dans l'urine de rats mâles Sprague-Dawley suivant une exposition répétée à des doses journalières de 1,25, 6,25 et 31,25 μmol de BaP/kg du mardi au vendredi pendant quatre semaines consécutives (Bouchard et Viau, 1995). Des collectes urinaires de 24 heures ont été effectuées les lundis (suite à 72 heures sans traitement) et les jeudis. Un profil en dents de scie a été observé, avec des maxima les jeudis et des minima les lundis. Une augmentation progressive de l'excrétion du lundi et du jeudi au fil des semaines était également observée. L'augmentation des valeurs du lundi dans le temps laissait supposer une augmentation probable de la charge corporelle en BaP ou ses métabolites au fil des semaines. L'augmentation de l'excrétion du 3-OHBaP dans l'urine du jeudi, au fil des semaines, serait probablement liée à une induction enzymatique du BaP et à une contribution de la charge croissante en BaP ou ses métabolites dans l'organisme.

Enfin, il a été observé que la fraction molaire de la dose de BaP excrétée sous forme de 3-OHBaP dans l'urine était plus faible que celle de pyrène excrétée sous forme de 1-OHP chez le rat. En effet, en comparant les résultats obtenus par Dodd et Viau (non publié) pour le 1-OHP et ceux obtenus dans une récente publication (Bouchard et Viau, 1995) pour le 3-OHBaP, il est possible d'estimer approximativement que le 1-OHP est excrété en quantité 50 fois plus grande que le 3-OHBaP pour une dose équivalente administrée. Il est à noter que les métabolites conjugués du BaP ont un poids moléculaire plus élevé que ceux du pyrène ce qui favorise une excrétion biliaire plus importante de ces premiers. Par exemple, les glucurono-conjugués du 3-OHBaP et du 1-OHP ont des poids moléculaires de 428 et 378, respectivement.

1.4.1.3. Influence de divers facteurs sur l'excrétion urinaire des métabolites du BaP chez l'animal

1.4.1.3.1. Variations selon la voie d'exposition

Dans des études chez l'animal, il a été démontré que l'excrétion urinaire de métabolites du BaP était influencée par la voie d'entrée du composé dans l'organisme (Jongeneelen *et al.*, 1984; Van de Wiel *et al.*, 1993). Jongeneelen *et al.* (1984) ont noté des différences dans le profil d'excrétion et dans les quantités excrétées du 3-OHBaP après administrations orale et cutanée chez le rat traité avec des doses de 10, 20 et 50 μmol de BaP/kg par jour pendant trois jours consécutifs. Aux faibles doses, l'excrétion urinaire du 3-OHBaP était plus élevée après exposition cutanée qu'après administration orale. Au contraire, à la plus forte dose, les niveaux d'excrétion après administration orale étaient plus élevés qu'après administration cutanée. Peu importe la dose, l'élimination du 3-OHBaP semblait toutefois plus rapide après administration orale qu'après application cutanée.

Van de Wiel *et al.* (1993) ont, pour leur part, observé des différences dans le pic d'excrétion et dans la quantité de métabolites du BaP excrétés dans l'urine après administrations orale et intrapéritonéale de 1 mmol de BaP/kg. Comme mentionné précédemment, l'excrétion maximale était atteinte aux jours deux et trois suivant une injection intrapéritonéale tandis que le pic d'excrétion était observé aux jours un et deux suivant une exposition orale. Les quantités totales de métabolites du BaP excrétés dans l'urine étaient en moyenne quatre fois plus élevées après injection intrapéritonéale qu'après administration orale chez les mâles. Au contraire, 2,4 fois moins de métabolites du BaP étaient retrouvés dans l'urine de rats femelles après injection intrapéritonéale qu'après exposition par

voie orale.

1.4.1.3.2. Variations selon l'espèce, la souche et le sexe

Des différences dans le profil quantitatif des divers métabolites du BaP dans l'urine sont apparentes selon l'espèce, la souche et le sexe. En effet, Likhachev *et al.* (1992) et Tyndyk *et al.* (1994) ont rapporté que le 7,8-diolBaP était excrété en quantité plus grande que le 3-OHBaP dans l'urine de rats LIO exposés, respectivement, à 200 mg de BaP/kg (793 $\mu\text{mol/kg}$) et 10 mg de BaP/kg (39,6 $\mu\text{mol/kg}$) par voie intrapéritonéale. Van de Wiel *et al.* (1993) ont mentionné, au contraire, que le 3-OHBaP était excrété en quantité 1000 fois supérieure au 7,8-diolBaP chez des rats Wistar mâles exposés de façon aiguë à 1 mmol de BaP/kg par voies orale et intrapéritonéale. Par ailleurs, ces derniers auteurs rapportèrent que, peu importe la voie d'administration, la fraction molaire de la dose excrétée sous forme de métabolites totaux dans l'urine était plus faible chez les rats femelles que chez les rats mâles.

En comparant les résultats des expériences de Van de Wiel *et al.* (1993) réalisées chez des rats Wistar à ceux des expériences de Camus *et al.* (1984) effectuées chez des souris C56BI/6 (B6) et DBA/2 (D2), des différences dans le pourcentage de la dose excrétée sous forme de divers métabolites majeurs du BaP dans l'urine sont apparentes. Les données de Van de Wiel *et al.* (1993) ont montré qu'approximativement jusqu'à 0,5% de la dose de BaP était excrété sous forme de 3-OHBaP dans l'urine alors que jusqu'à 0,005% de la dose était récupéré sous forme de 4,5-diolBaP. Au contraire, selon Camus *et al.* (1984), 0,002 à 0,009% et 0,001 à 0,002% d'une dose de 100 mg de BaP/kg (396

$\mu\text{mol/kg}$) par voie intrapéritonéale était excrété sous forme de 3-OHBP et 4,5-diolBP, respectivement. Ces derniers auteurs rapportèrent de plus que les ratios de concentrations urinaires entre le 3-OHBP, 4,5-diolBP et 7,8-diolBP variaient en fonction de la souche de souris. Ils observèrent que, chez les souris (B6) inductibles, les quantités relatives de métabolites dans l'urine étaient: 3-OHBP > 7,8-diolBP > 4,5-diolBP et au contraire, chez les souris (D2) non inductibles, suivaient plutôt l'ordre: 7,8-diolBP > 3-OHBP \approx 4,5-diolBP. Ces dernières études soulignent donc qu'il existe, non seulement des différences dans l'excrétion urinaire des métabolites du BP en fonction de la voie d'exposition, mais également des variations dans l'excrétion selon l'espèce, la souche et le sexe.

En résumé, la mesure des métabolites du BP dans l'urine présente a priori un intérêt pour la surveillance biologique de l'exposition aux HAP étant donné le potentiel cancérigène du BP. Néanmoins, ces métabolites sont peu ou pas mesurables dans l'urine humaine avec les méthodes analytiques courantes. Leur utilisation comme bioindicateur de l'exposition aux HAP chez l'humain nécessitera la mise au point et la validation de méthodes analytiques très sensibles. D'autre part, les divergences dans les données de la littérature animale suggèrent qu'une étude plus systématique de l'effet de la dose, de la voie d'exposition et du moment d'échantillonnage sur l'excrétion urinaire des métabolites du BP devrait être effectuée. La cinétique d'excrétion urinaire des métabolites du BP devrait en outre être établie de façon plus précise.

1.4.2. Le 1-OHP urinaire

1.4.2.1. Importance et utilité

Contrairement aux métabolites du BaP, le 1-OHP, qui comme indiqué précédemment est le métabolite principal du pyrène, est facilement mesuré dans l'urine humaine, même chez des populations témoins (Viau *et al.*, 1995b). En effet, Jongeneelen *et al.* (1987) ont développé une méthode d'analyse simple et sensible pour la détermination du 1-OHP dans l'urine humaine qui, comme mentionné précédemment, consiste en l'hydrolyse enzymatique des glucurono- et sulfo-conjugués du 1-OHP, extraction en phase solide et analyse par CLHP/fluorescence. Cette méthode a d'ailleurs été recommandée pour la surveillance biologique de l'exposition aux HAP (Jongeneelen et Anzion, 1991) et la plupart des laboratoires effectuant ce type de mesures l'ont adoptée ou en ont fait une adaptation. Récemment, d'autres méthodes sensibles qui consistent à mesurer le conjugué glucuronide du 1-OHP ont également été développées (Kang *et al.*, 1995; Singh *et al.*, 1995)

Étant donné l'importante proportion du pyrène dans la plupart des mélanges de HAP (Buchet *et al.*, 1992; Roussel *et al.*, 1992) et la facilité de mesurer le 1-OHP dans l'urine humaine (Hansen *et al.*, 1993a; Jongeneelen *et al.*, 1985a; 1986; 1990), plusieurs chercheurs se sont intéressés à la mesure de ce dernier comme indicateur biologique de l'exposition aux HAP en milieu de travail. En fait, la majorité des études humaines s'intéressant à la mesure des métabolites hydroxylés de HAP spécifiques dans l'urine ont porté sur le 1-OHP et les données disponibles à l'heure actuelle sur le 1-OHP portent en grande partie sur des études effectuées chez des travailleurs. Jongeneelen *et al.* (1987) montrèrent qu'il était possible d'estimer l'importance de l'exposition à des HAP en s'appuyant sur la mesure de

ce métabolite dans l'urine. Il a été possible d'établir des corrélations entre, d'une part, les concentrations de HAP totaux dans divers médias et celles du pyrène (Jongeneelen *et al.*, 1990; Tolos *et al.*, 1990; Van Rooij, 1994) et, d'autre part, entre les concentrations de HAP dans l'air des milieux de travail, exprimées sous forme de matières solubles dans le benzène (MSB), de HAP totaux ou de HAP individuels tels que le BaP et le pyrène, et celles du 1-OHP dans l'urine de travailleurs (Kuljukka *et al.*, 1996; Tolos *et al.*, 1990; Zhao *et al.*, 1990; 1992a). Malgré la publication de plusieurs études sur l'utilisation du 1-OHP comme bioindicateur de l'exposition, on reste toutefois étonné devant la pauvreté des données toxicocinétiques fondamentales, essentielles à une interprétation juste des mesures réalisées chez des populations exposées principalement par les voies pulmonaire et cutanée pour les travailleurs et par les voies pulmonaire et digestive pour la population générale. Comme mentionné précédemment, la connaissance approfondie du comportement toxicocinétique du pyrène et de son métabolite est d'autant plus importante que la mesure urinaire du 1-OHP est utilisée, dans des enquêtes épidémiologiques, comme indice de l'exposition à l'ensemble des HAP chez les travailleurs ou la population générale. Dans le contexte de telles études, il n'est en effet habituellement possible de recueillir que des prélèvements urinaires ponctuels, non minutés. Il importe dès lors de déterminer le moment le plus adéquat pour le prélèvement de l'échantillon biologique.

1.4.2.2. Cinétique de l'excrétion urinaire du 1-OHP

1.4.2.2.1 Études réalisées chez des travailleurs

La plupart des publications portant sur la mesure de l'excrétion urinaire du 1-OHP chez des individus

exposés aux HAP en milieu de travail sont de type descriptif. L'évaluation de l'excrétion du 1-OHP chez des travailleurs au cours d'une journée ou d'une semaine de travail se rapporte essentiellement à une ou deux mesures urinaires ponctuelles du métabolite le matin, l'après-midi et/ou le soir, en début et/ou en fin de semaine de travail. Ces données n'apportent donc qu'une estimation grossière du comportement cinétique du 1-OHP dans l'urine. Elles ont toutefois démontré que les concentrations de ce métabolite étaient plus élevées chez les individus exposés aux HAP en milieu de travail que dans des populations témoins ou de référence, que l'excrétion du 1-OHP était influencée par des facteurs confondants tels que le tabagisme et qu'elle variait selon le milieu de travail et selon de poste de travail dans un milieu donné (Angerer *et al.*, 1997; Boogaard and Van Sittert, 1995; Burgaz *et al.*, 1992; Clonfero *et al.*, 1995; Göen *et al.*, 1995; Grimmer *et al.*, 1991; Jongeneelen *et al.*, 1989; Levin *et al.*, 1995; Ovrebo *et al.*, 1994; 1995a; Viau *et al.*, 1993; Zhao *et al.*, 1990; 1992b). Par ailleurs, dans plusieurs de ces études, l'excrétion du 1-OHP dans des collectes de début et de fin de quart de travail ou de début et de fin de semaine de travail a été comparée et ceci a permis de mettre en évidence que les concentrations de 1-OHP variaient pendant la journée de travail et au cours de la semaine de travail. En général, les valeurs étaient plus élevées dans une collecte de fin de journée de travail qu'avant le début de celle-ci (Boogaard *et al.*, 1995; Elovaara *et al.*, 1992; 1995; Ferreira, Jr. *et al.*, 1994; Jongeneelen *et al.*, 1988c; Malkin *et al.*, 1996; Tolos *et al.*, 1990; Vanhummelen *et al.*, 1993; Van Rooij *et al.*, 1993a) et les concentrations étaient plus élevées dans une collecte de fin de semaine de travail qu'au début de la semaine (Elovaara *et al.*, 1992; 1995; Granella et Clonfero, 1993; Ny *et al.*, 1993; Omland *et al.*, 1994; Van Rooij *et al.*, 1994a).

Certains auteurs ont toutefois effectué un examen un peu plus détaillé du profil d'excrétion urinaire

du 1-OHP dans le temps chez des travailleurs à partir de collectes effectuées avant et/ou pendant ou après le travail pendant toute la semaine de travail (Bos et Jongeneelen, 1988; Jongeneelen *et al.*, 1985a; 1988a,b; Petry *et al.*, 1996; Quinlan *et al.*, 1995a; Van Rooij *et al.*, 1993a; Van Schooten *et al.*, 1995). Dans certaines publications, des collectes ont également été effectuées une ou deux fois par jour pendant une fin de semaine de congé ou une période de répit de quelques jours (Buchet *et al.*, 1992; Jongeneelen *et al.*, 1985a; 1988a; Quinlan *et al.*, 1995a,b) ou pendant une période plus longue de vacances (Jongeneelen *et al.*, 1988a). Un nombre restreint d'auteurs ont également recueilli toutes les mictions au cours d'une journée de travail pendant plusieurs jours de travail incluant la fin de semaine (Elovaara *et al.*, 1995; Heikkilä *et al.*, 1995; Van Rooij *et al.*, 1993c).

Ces dernières études ont démontré que l'excrétion du 1-OHP dans l'urine augmentait au cours de la journée de travail et que les valeurs maximales étaient atteintes de trois à neuf heures après la fin du quart de travail. Dans la plupart des publications, une augmentation nette de l'excrétion du 1-OHP pendant la période de travail a été observée en recueillant les urines avant et à la fin du quart. Par contre, dans les milieux de travail où l'absorption cutanée est importante tels que les usines d'imprégnation du bois à la créosote, il a été rapporté que lorsque les travailleurs étaient exposés le jour précédant l'échantillonnage, les concentrations de 1-OHP dans l'urine de fin du quart de travail pouvaient être plus faibles que dans les urines recueillies avant le début du quart (Elovaara *et al.*, 1995). Lorsque les travailleurs n'étaient pas exposés le jour précédant l'échantillonnage, les valeurs d'excrétion étaient toutefois plus élevées dans les urines de fin de quart de travail que dans les urines de début de quart. Ce phénomène a été attribué au fait que l'absorption cutanée se prolongeait probablement après les heures de travail. Il est par contre important de noter que dans toutes les

situations, le pic d'excrétion journalier était atteint en soirée.

Par ailleurs, ces études où plusieurs échantillons d'urine ont été recueillis ont permis d'observer que les valeurs d'excrétion du 1-OHP augmentaient pendant les trois premiers jours de la semaine de travail et demeuraient constantes les jours de travail suivants (Jongeneelen *et al.*, 1985a; 1988a; Van Rooij *et al.*, 1993a). L'excrétion du 1-OHP diminuait pendant la fin de semaine ou un congé de plusieurs jours, sans toutefois qu'elle n'atteigne pour les plus exposés, le matin du retour au travail, les niveaux de base de personnes non exposées en milieu de travail (Jongeneelen *et al.*, 1985a; 1988a), suggérant ainsi une certaine bioaccumulation dans l'organisme humain. Il a également été rapporté que les variations dans l'excrétion du 1-OHP d'une semaine à l'autre étaient faibles (Quinlan *et al.*, 1995b).

Certaines études ont de plus permis de calculer une demi-vie d'élimination du 1-OHP dans l'urine. Jongeneelen *et al.* (1988a) observèrent deux phases d'élimination avec des demi-vies de un à deux jours et de 16 jours chez un travailleur d'une usine d'imprégnation du bois à la créosote en recueillant les urines pendant une fin de semaine de congé et une période de vacances de 17 jours, respectivement. La première phase correspondrait à la charge corporelle rapidement disponible (c'est-à-dire le compartiment central et les tissus en équilibre direct avec celui-ci) tandis que la deuxième phase correspondrait à une composante lente, soit l'élimination du pyrène stocké dans les tissus faiblement perfusés tels que les tissus gras. Ces valeurs de demi-vies s'appuyaient toutefois sur un faible nombre d'échantillons et d'individus ne permettant qu'une détermination grossière des paramètres cinétiques.

Jongeneelen *et al.* (1990) ont estimé, par ailleurs, une demi-vie de six à 35 heures pour la phase initiale d'élimination chez 18 travailleurs de fours à coke. Récemment, Boogaard et Van Sittert (1994) ont déterminé une demi-vie moyenne de 10,4 heures pour cette phase initiale d'élimination chez 16 travailleurs de l'industrie pétrochimique. Malgré le nombre important d'individus, ces estimations de la demi-vie provenaient d'un très faible nombre de collectes. Quinlan *et al.* (1995b) ont estimé une demi-vie d'élimination moyenne de 19 heures chez cinq travailleurs d'une usine de liquéfaction du charbon en recueillant les urines de début de matinée pendant une période de congé de quatre jours. Buchet *et al.* (1992) rapportèrent une valeur moyenne de demi-vie d'environ 18 heures à partir de collectes urinaires effectuées à la fin d'une semaine de travail et à divers moments pendant une période de congé de quatre jours (environ six à sept collectes ont été effectuées pendant cette période) chez 15 travailleurs d'une usine d'électrodes de graphite. Cette valeur de demi-vie, qui correspond à la première phase d'élimination décrite par Jongeneelen *et al.* (1988a), apparaît plus fiable puisque celle-ci s'appuie sur un nombre de prélèvements et un nombre d'individus plus important. Ces valeurs de demi-vies sont, par ailleurs, en accord avec une augmentation de l'excrétion de 1-OHP pendant la semaine de travail. En effet, une demi-vie de 10,4, 18 ou 19 heures ou de un à deux jours implique qu'un travailleur n'a pas le temps d'excréter tout le 1-OHP formé entre la fin d'une journée de travail et le début de la journée de travail suivante.

1.4.2.2.2. *Études réalisées chez des volontaires humains*

Dans une étude alimentaire semi-contrôlée, Buckley et Liroy (1992) évaluèrent le profil d'excrétion du 1-OHP dans l'urine chez cinq volontaires ayant ingéré un mets riche en HAP. Une demi-vie

d'élimination apparente de premier ordre de 4,4 heures a été estimée à partir de collectes effectuées à intervalles de huit heures. Les prélèvements n'étaient cependant pas assez fréquents pour permettre d'évaluer une demi-vie fiable.

Récemment, le profil cinétique détaillé d'excrétion urinaire du 1-OHP (prélèvements à intervalles de 0,5 à 4 heures) a été établi chez deux volontaires exposés à une dose aiguë de pyrène par voies cutanée et orale (Viau *et al.*, 1995a). L'examen visuel du profil suggérait au moins deux phases d'élimination. Une demi-vie apparente de premier ordre d'environ 12 heures, pour la phase comprise entre 12 et 48 heures après exposition, a été estimée après administration par l'une ou l'autre voie. Les deux phases d'élimination ne correspondent pas aux mêmes phases que celles décrites par Jongeneelen *et al.* (1988a). En effet, la seconde phase décrite par ces derniers auteurs correspondait à l'élimination d'une charge corporelle découlant d'une exposition professionnelle alors que la charge corporelle en pyrène des volontaires ne correspondait qu'au bruit de fond de la contamination environnementale. Signalons en terminant que dans les 48 heures suivant l'administration, la proportion molaire de pyrène administré se retrouvant dans l'urine sous forme de 1-OHP atteignait 3,7% suivant l'administration par voie orale et 0,17% après administration par voie cutanée.

Viau et Vyskocil (1995) ont également déterminé la cinétique détaillée d'excrétion urinaire du 1-OHP chez un patient psoriasique se traitant avec un shampoing à base de goudron de houille. En recueillant toutes les urines pendant environ 80 heures suivant le traitement, un patron d'excrétion semblable à celui obtenu chez les volontaires humains exposés au pyrène (Viau *et al.*, 1995a) a été observé et il a été possible de calculer une demi-vie d'élimination fiable de 15 heures. De plus, la

cinétique détaillée d'excrétion urinaire du 1-OHP chez deux volontaires après application cutanée de 100 µL de créosote sur la face interne de l'avant bras a été examinée. Toutes les urines ont été recueillies sur une période de 48 heures. À l'examen visuel, le profil cinétique d'excrétion comprenait deux phases et une demi-vie d'élimination de 12 heures environ a été obtenue pour la phase comprise entre 12 et 48 heures tout comme après administration du pyrène seul.

Jongeneelen *et al.* (1985a; 1986; 1988b) ont précédemment examiné la cinétique d'excrétion urinaire du 1-OHP chez des patients psoriasiques se traitant de façon journalière avec des onguents à base de goudron de houille. Des collectes urinaires avaient été effectuées avant le traitement et matin et soir pendant trois jours. Ces auteurs observèrent une augmentation très rapide de l'excrétion du 1-OHP et des niveaux élevés se maintenaient pendant toute la période de traitement. Aucune demi-vie apparente d'élimination de premier ordre n'a pu être déterminée, étant donné que le traitement quotidien se poursuivait pendant toute la période de collectes.

Van Rooij *et al.* (1993b) ont, par contre, examiné la cinétique d'excrétion urinaire détaillée du 1-OHP chez des volontaires après application cutanée d'un onguent à base de goudron de houille. Toutes les urines ont été recueillies pendant les 24 heures avant le traitement et dans les 72 heures suivant l'application. Après exposition, les niveaux de 1-OHP atteignaient six à dix fois l'excrétion de base avant traitement. Une demi-vie d'élimination variant entre 8,2 et 18,9 heures a été rapportée, celle-ci variant significativement entre les volontaires et selon le site d'application.

Van Schooten *et al.* (1994) ont pour leur part étudié le comportement cinétique grossier du 1-OHP

chez 11 individus après traitement avec un shampoing à base de goudron de houille par rapport à des contrôles. Cependant le nombre de collectes limité ne permettait pas l'estimation des paramètres cinétiques.

Étant donné la demi-vie d'élimination apparente de premier ordre de 12 heures obtenue chez des volontaires humains sans exposition professionnelle (Viau et Vyskocil, 1995; Viau *et al.*, 1995a) ou de 8,2 à 18,9 heures (Van Rooij *et al.*, 1993b), on peut s'attendre à une accumulation du 1-OHP dans l'urine suivant une exposition répétée au pyrène ou au goudron de houille. Viau et Vyskocil (1995) observèrent, en effet, une augmentation progressive des maxima et minima d'excrétion urinaire du 1-OHP chez des volontaires exposés une fois par jour au pyrène pendant cinq jours consécutifs par voie cutanée. Un plateau semblait être atteint après trois jours ce qui est en accord avec la demi-vie de 12 heures qui impliquerait qu'après trois jours, la contribution de la première application serait négligeable. Par ailleurs, suite à l'arrêt de l'exposition, deux phases d'élimination étaient observées: une première phase plus rapide et une seconde phase plus lente.

1.4.2.2.3. Études réalisées chez l'animal

Les données sur la cinétique d'excrétion urinaire du 1-OHP chez les animaux concernent majoritairement des expositions aiguës. Jongeneelen *et al.* (1985a) ont étudié le profil cinétique grossier de l'excrétion du 1-OHP dans l'urine suivant une administration unique de diverses doses de pyrène (0,05, 0,5, 5, 50 et 100 µg par rat ou 0,25, 2,5, 24,7, 247 et 494 nmol par rat) par voie orale chez le rat. Ces auteurs ont montré que la quantité de 1-OHP dans l'urine était fonction de la

dose d'exposition et augmentait avec l'accroissement de la dose. L'excrétion de 1-OHP s'était accrue rapidement après l'exposition et l'élimination était complète, ou presque, en 96 heures. Néanmoins, les collectes étaient effectuées à intervalles de 24 heures pendant quatre jours après traitement, de sorte qu'il n'était pas possible d'estimer une demi-vie d'élimination fiable.

Selon un protocole semblable, Jongeneelen *et al.* (1986) ont exposé des rats Wistar mâles à une dose unique de 0, 2,5, 12,6 et 53,0 mg de goudron de houille par voie cutanée. Le 1-OHP a été quantifié dans des collectes urinaires de 24 heures effectuées sur une période de 120 heures (cinq jours). L'excrétion du 1-OHP augmentait de façon proportionnelle à la dose. L'excrétion maximale était atteinte dans les 24 premières heures suivant l'application des deux plus faibles doses tandis que, pour la plus forte dose, le pic d'excrétion était atteint entre 24 et 48 heures après le traitement. Par la suite, une diminution progressive des quantités excrétées de 1-OHP dans l'urine était observée. L'élimination était complète ou presque en 120 heures. Toutefois, le profil cinétique détaillé de l'excrétion ne fut pas examiné dans cette étude de sorte que les renseignements qu'elle apporte sont, encore une fois, limités.

Jacob *et al.* (1989) ont examiné le profil grossier d'excrétion urinaire du 1-OHP suivant une exposition aiguë au pyrène par diverses voies d'administration. Ces auteurs ont exposé des rats à 50 µg de pyrène (0,25 µmol) par voies orale et intrapéritonéale, à 1 mg (4,9 µmol) par voie intrapulmonaire ou à 400 et 800 ng (1,98 et 3,96 nmol) par voie intratrachéale. En effectuant des collectes de 24 heures pendant trois ou quatre jours après l'administration, ils montrèrent que la cinétique grossière d'élimination apparaissait plus ou moins semblable après administrations orale et

intrapéritonale et les valeurs semblaient chuter plus rapidement dans le temps qu'après instillation intrapulmonaire. Les niveaux d'excrétion après instillation intratrachéale étaient trop faibles pour tirer des conclusions quant au comportement cinétique. Néanmoins, peu importe la voie d'entrée du contaminant dans l'organisme, l'élimination était presque complète en trois jours. Cette étude ne nous renseigne toutefois pas davantage sur le profil d'excrétion détaillé au cours des 24 ou 48 heures suivant l'exposition.

Soulignons que dans une expérience récente, le profil temporel détaillé d'excrétion urinaire du 1-OHP a été étudié chez le rat suivant une exposition aiguë au pyrène (de 0.5 à 50 $\mu\text{mol/kg}$) par différentes voies d'administration (orale, intraveineuse et cutanée) (Dodd et Viau, non publié). Le profil cinétique d'excrétion était semblable à celui que nous avons observé chez des volontaires humains (Viau *et al.*, 1995a). Le pic d'excrétion était atteint en moins d'une heure après injection intraveineuse, entre deux et cinq heures après administration orale et 10 à 12 heures après application cutanée. L'élimination était biphasique peu importe la voie d'administration et la demi-vie moyenne apparente de premier ordre calculée à partir des données d'excrétion entre 12 heures et 48 heures après exposition était, respectivement, de 6,3, 7,0 et 8,6 heures pour les voies orale, intraveineuse et cutanée. Les similitudes dans le comportement cinétique du 1-OHP dans l'urine de rats et d'humains laissent supposer que l'animal peut servir de modèle adéquat pour l'étude de la toxicocinétique du 1-OHP. Par ailleurs, au cours des 48 heures suivant l'administration, la proportion molaire de pyrène excrétée dans l'urine sous forme de 1-OHP atteignait respectivement 0,36, 0,70 et 0,26% en moyenne pour les voies orale, intraveineuse et cutanée. Les valeurs plus faibles après administration cutanée s'expliquent par le fait que la peau agit comme barrière protectrice limitant l'absorption du pyrène.

D'un autre côté, Weyand *et al.* (1991) ont examiné l'excrétion urinaire du 1-OHP chez des souris B6C3F1 suivant l'ingestion de goudron de houille pendant 15 jours. Une collecte de 16 heures a été effectuée le premier et dernier jour d'ingestion et une augmentation de l'excrétion du 1-OHP a été observée entre ces deux périodes de collectes. En terminant, Kanoh *et al.* (1993) ont également étudié la cinétique d'excrétion du 1-OHP dans l'urine de rats suivant une exposition à des doses répétées de pyrène par inhalation ou par ingestion pendant huit semaines. Ils ont démontré une augmentation, par rapport aux contrôles, des niveaux de 1-OHP dans l'urine au fil des semaines d'exposition pour des collectes effectuées après deux, quatre et huit semaines de traitement. Encore une fois, l'information disponible sur la cinétique détaillée d'excrétion urinaire du 1-OHP chez des animaux exposés de façon subchronique (plusieurs semaines) est très limitée et mérite d'être mieux documentée.

1.4.2.3. Influence de divers facteurs sur l'excrétion urinaire du 1-OHP

1.4.2.3.1. La voie d'exposition au pyrène

1.4.2.3.1.1. Études réalisées chez l'humain

Bien que l'inhalation soit souvent considérée comme la voie majeure d'exposition aux HAP en milieu de travail, la contribution de l'exposition cutanée peut également être importante dans certains types de travail, notamment dans les unités de production d'anodes pré-cuites des usines d'aluminium ou encore chez les travailleurs de fours à coke, dans l'industrie pétrochimique et chez les individus exposés à la créosote (Boogaard et Van Sittert, 1994; Van Rooij *et al.*, 1992; 1993a,c).

Van Rooij *et al.* (1992) ont montré que les coefficients de corrélation entre la contamination cutanée et les concentrations de 1-OHP dans des urines de fin de quart de travail chez des travailleurs de l'industrie d'aluminium primaire étaient égaux ou plus élevés que ceux établis entre la concentration de pyrène dans l'air et le 1-OHP dans l'urine. Ces auteurs estimèrent que la contamination par la peau était plus de trois fois supérieure à l'apport par voie respiratoire et donc qu'elle pourrait contribuer considérablement à la charge corporelle de pyrène chez un travailleur.

Dans une autre étude chez des travailleurs de fours à coke, Van Rooij *et al.* (1993a) rapportèrent, en estimant les doses cutanée et respiratoire, qu'en moyenne 75% (28-95%) et 51% (8-92%) de la quantité totale de pyrène et de BaP absorbée par l'organisme, respectivement, pénétrerait dans l'organisme par la peau. En effet, les variations dans l'excrétion urinaire du 1-OHP au cours d'une semaine de travail étaient davantage déterminées par la dose cutanée de pyrène que par la dose respiratoire. Ces auteurs conclurent que, chez les travailleurs de fours à coke, la voie cutanée est la voie majeure d'absorption des HAP.

Chez des travailleurs exposés à la créosote dans une usine d'imprégnation du bois, Van Rooij *et al.* (1993c) observèrent une bonne corrélation entre l'exposition par la peau et l'excrétion urinaire du 1-OHP tandis que les concentrations de pyrène dans l'air étaient moins bien reliées à l'excrétion du 1-OHP. Ces auteurs rapportèrent que le port d'habits protecteurs, bien que n'étant pas très efficace (probablement dû à certaines parties non recouvertes), réduisait significativement la quantité de 1-OHP excrétée et diminuait de 35%, en moyenne, la contamination de pyrène sur la peau. Selon eux, un lavage plus fréquent des habits et une amélioration de l'hygiène personnelle conduirait

probablement à une réduction de la dose interne de HAP. Ces auteurs affirmèrent que la contribution de la dose inhalée était trop faible pour expliquer l'importance de l'excrétion urinaire du 1-OHP et suggérèrent une contribution probable de l'absorption cutanée.

Boogaard et Van Sittert (1994) rapportèrent également que la contamination cutanée, tout comme l'exposition par inhalation, contribuait significativement à l'exposition aux HAP chez des travailleurs de l'industrie pétrochimique et que l'importance de l'exposition cutanée variait selon le poste de travail. Par ailleurs, Quinlan *et al.* (1995b) calculèrent que l'exposition cutanée contribuait à plus de 70% de l'excrétion urinaire du 1-OHP chez les travailleurs d'une usine de liquéfaction du charbon en comparant l'excrétion urinaire totale du 1-OHP (aire sous la courbe des taux d'excrétion en fonction du temps) avec la contribution des concentrations de pyrène dans l'air ambiant.

De ces études en milieu de travail, il est toutefois difficile d'évaluer l'influence de l'absorption cutanée sur la cinétique d'excrétion du 1-OHP dans l'urine. Cependant, comme mentionné précédemment, Elovaara *et al.* (1995) rapportèrent que l'exposition cutanée pouvait influencer le profil d'excrétion urinaire du 1-OHP au cours d'une semaine de travail chez des individus exposés. Par ailleurs, Viau *et al.* (1995a) comparèrent l'excrétion urinaire du 1-OHP chez des volontaires après administrations orale et cutanée de pyrène. Bien que, pour une même dose d'exposition, l'excrétion après administration orale était beaucoup plus élevée qu'après administration cutanée, l'absorption cutanée restait tout de même appréciable. Il est par ailleurs intéressant de noter qu'en traçant la cinétique détaillée d'excrétion par ces deux voies, la phase tardive de la courbe d'élimination était parallèle pour les deux voies d'administration considérées et une demi-vie d'environ 12 heures a été calculée.

1.4.2.3.1.2. *Études réalisées chez l'animal*

Jacob *et al.* (1989) ont étudié l'effet de la voie d'administration du pyrène sur l'excrétion urinaire du 1-OHP chez le rat. Ils ont rapporté des différences dans la quantité de 1-OHP excrétée dans l'urine selon la voie d'entrée du contaminant dans l'organisme. En effet, les pourcentages de la dose excrétée dans l'urine sous forme de 1-OHP étaient comparables après administrations orale et intrapéritonéale. Ces valeurs étaient beaucoup plus élevées qu'après administration intrapulmonaire et encore plus élevées qu'après administration intratrachéale. Comme il a déjà été mentionné, selon l'étude de Dodd et Viau (non publié), le pic d'excrétion urinaire du 1-OHP chez le rat et la fraction molaire de la dose excrétée sous forme de 1-OHP dans l'urine dépendaient de la voie d'administration. Toutefois, peu importe la voie d'administration, la cinétique d'élimination était biphasique et des demi-vies comparables ont été calculées pour la phase terminale d'élimination après administrations intraveineuse, orale et cutanée.

1.4.2.3.2. *La fumée de cigarette*

Chez les individus avec une faible exposition environnementale aux HAP, la consommation de tabac cause, en général, une augmentation significative de l'excrétion urinaire du 1-OHP (Van Rooij *et al.*, 1994b). Cependant, à mesure que les niveaux d'exposition provenant d'autres sources s'accroissent, les différences dans l'excrétion urinaire du 1-OHP entre fumeurs et non-fumeurs disparaissent (Ovrebo *et al.*, 1995b). En effet, Levin *et al.* (1995a,b) ont rapporté que l'excrétion urinaire de base du 1-OHP chez des individus non exposés de façon professionnelle était influencée par la fumée de

cigarette et que, normalement, la différence dans les niveaux de 1-OHP entre les fumeurs et non-fumeurs était statistiquement significative. Goën *et al.* (1995) affirmèrent d'ailleurs que la fumée de cigarette était le facteur qui influençait en majeure partie l'excrétion du 1-OHP dans la population générale. Levin (1995a) nota par ailleurs que les niveaux urinaires de 1-OHP chez les fumeurs non exposés en milieu de travail étaient du même ordre de grandeur que ceux de travailleurs faiblement exposés aux HAP dans divers milieux professionnels.

Plusieurs auteurs ont toutefois noté, comme mentionné précédemment, que chez des travailleurs exposés à de fortes concentrations en HAP, la contribution de la cigarette était négligeable. En effet, Buchet *et al.* (1992) rapportèrent que la contribution relative de la fumée de tabac était négligeable en comparaison avec l'exposition aux HAP en milieu professionnel chez des travailleurs d'une manufacture d'électrodes de graphite. De façon similaire, Tolos *et al.* (1990) n'ont pas observé de contribution importante de la consommation de tabac sur l'excrétion urinaire du 1-OHP chez des travailleurs d'une usine de production d'aluminium. Burgaz *et al.* (1992) rapportèrent que l'excrétion de 1-OHP était influencée par la consommation de cigarette mais que la contribution de l'exposition professionnelle était beaucoup plus importante. Elovaara *et al.* (1995) affirmèrent également que, même si la fumée de cigarette est connue pour augmenter les concentrations de 1-OHP, ce facteur apparaissait être une source négligeable de pyrène chez les travailleurs de la créosote.

Il reste que Van Schooten *et al.* (1995) ont observé des concentrations urinaires plus élevées de 1-OHP chez les fumeurs par rapport aux non-fumeurs autant chez les travailleurs de l'aluminium faiblement que fortement exposés. Une relation claire a également été établie entre le 1-OHP dans

l'urine et le nombre de cigarettes fumées par jour. Tout comme Jongeneelen *et al.* (1990) et Mielżyńska *et al.* (1997) ont observé chez des travailleurs de fours à coke, ces auteurs ont noté que la différence entre les niveaux d'excrétion du 1-OHP chez les fumeurs et non-fumeurs était plus prononcée dans les groupes avec la plus forte exposition aux HAP. Ils suggérèrent un effet synergique de la fumée de cigarette en combinaison avec l'exposition aux HAP en milieu de travail sur l'excrétion urinaire du 1-OHP.

Certains auteurs n'ont toutefois pas observé d'augmentation statistiquement significative dans l'excrétion urinaire du 1-OHP chez les sujets contrôles fumeurs par rapport aux non-fumeurs. Omland *et al.* (1994) rapportèrent que la fumée de cigarette n'affectait pas les concentrations urinaires de 1-OHP autant chez les travailleurs de fonderies du fer que chez la population contrôle. Jongeneelen *et al.* (1988a,b) rapportèrent également que les niveaux de 1-OHP chez les sujets contrôles fumeurs n'étaient pas statistiquement plus élevés que chez les contrôles non-fumeurs. En terminant, Zhao *et al.* (1992b) mentionnèrent que la différence dans l'excrétion du 1-OHP chez des volontaires fumeurs et non-fumeurs de la ville de Beijing n'était pas significative. Bien que les différences ne soient pas statistiquement significatives, toutes ces études montrent malgré tout des concentrations moyennes plus élevées du 1-OHP dans l'urine chez les sujets contrôles fumeurs que chez les individus contrôles non-fumeurs.

1.4.2.3.3. La nourriture riche en HAP

Plusieurs études ont montré que, tout comme la fumée de tabac, la consommation de HAP par la

nourriture peut mener à des variations interindividuelles dans l'excrétion urinaire de base du 1-OHP chez des individus non exposés en milieu de travail. En effet, Van Rooij *et al.* (1994b) ont montré un effet de la consommation de nourriture contenant des HAP sur l'excrétion urinaire de 1-OHP chez des volontaires. Ils notèrent que la consommation d'aliments contenant des HAP et la fumée de tabac comptaient pour 99% de l'absorption de pyrène chez des individus non exposés de façon professionnelle. Ceci confirme les résultats d'une étude, réalisée par Buckley *et al.* (1992) chez des volontaires, qui a démontré qu'une alimentation riche en HAP entraînait une augmentation de l'excrétion urinaire du 1-OHP.

Van Maanen *et al.* (1994) ont également montré que la consommation abondante de viande grillée contenant un haut niveau de BaP et de pyrène (8,6 et 26,5 µg/kg ou 34,1 et 131 nmol/kg, respectivement) (soit deux hamburgers par jour (170 g) pendant cinq jours) menait à une augmentation de l'excrétion urinaire du 1-OHP chez des volontaires. Par contre, la consommation de hamburgers avec de faibles niveaux en BaP et en pyrène ne causait aucune augmentation de l'excrétion du 1-OHP. Ces auteurs conclurent que la consommation orale de HAP pourrait accroître de façon dose-dépendante les niveaux de 1-OHP dans l'urine. Chénier et Viau (1997) n'ont pu mettre en évidence de relation entre l'apport alimentaire en HAP et l'excrétion urinaire de 1-OHP chez huit volontaires sans exposition professionnelle et non-fumeurs ayant rempli un journal alimentaire et ayant fourni toutes leurs mictions urinaires pendant cinq jours consécutifs. Les quantités de pyrène ingérées étaient peut-être trop faibles pour influencer l'excrétion urinaire du 1-OHP. Le journal alimentaire n'était peut-être pas dûment rempli et le système de classification et de cote des aliments selon leur contenu en pyrène n'était peut-être pas adéquat.

Il ressort donc que pour des expositions environnementales faibles comme chez les populations non exposées en milieu de travail et constituant les groupes contrôles, la nourriture riche en pyrène peut généralement avoir un effet sur l'excrétion urinaire du 1-OHP. Cependant, lorsque l'exposition environnementale est importante comme en milieu de travail, la contribution de la nourriture riche en HAP à l'excrétion urinaire du 1-OHP est faible (Kang *et al.*, 1995). Granello et Clonfero (1993) rapportèrent en effet que l'influence de la nourriture riche en HAP sur les niveaux d'excrétion du 1-OHP dans l'urine de travailleurs de la réparation automobile n'était détectable que lorsque les sujets retournaient au travail le lundi matin après une fin de semaine de congé (c'est-à-dire une collecte avant le quart de travail au début de la semaine de travail a été effectuée).

1.4.2.3.4. Autres facteurs: facteurs exogènes et facteurs d'hôte

Outre l'exposition par la fumée de cigarette et par la nourriture, on peut penser à l'effet d'autres facteurs exogènes ou facteurs d'hôte. Parmi les facteurs exogènes on peut noter un effet de la saison sur l'excrétion urinaire de base du 1-OHP chez des individus sans exposition professionnelle. En effet, Zhao *et al.* (1992a) ont observé des différences statistiquement significatives dans l'excrétion urinaire de 1-OHP chez des résidents de Beijing pendant la période hivernale par rapport à la période estivale. Ces auteurs suggérèrent que les niveaux d'excrétion plus élevés observés l'hiver chez les résidents étaient probablement dus au chauffage intérieur par brûlage de charbon. Par contre, chez des travailleurs d'une usine d'acier, ces variations dans l'excrétion du 1-OHP entre l'été et l'hiver n'étaient pas statistiquement significatives.

Zhao *et al.* (1992b) ont également mentionné un effet de l'heure de collectes sur l'excrétion urinaire de base chez des volontaires sans exposition professionnelle. Les niveaux maximum étaient retrouvés tard en soirée entre 21 et 23 heures. Dans des collectes effectuées pendant trois jours consécutifs, ils n'observèrent cependant pas de variations statistiquement significatives dans l'excrétion du 1-OHP d'un jour à l'autre.

Ces auteurs examinèrent également l'effet de facteurs comme l'âge et le sexe mais n'observèrent aucune relation entre ceux-ci et l'excrétion urinaire de 1-OHP. Jongeneelen *et al.* (1994) rapportèrent également que le sexe n'influait pas les niveaux d'excrétion du 1-OHP chez des enfants de la ville de Bytom dans la région de la Pologne. Enfin, Burgaz *et al.* (1992) montrèrent, pour leur part, que les médicaments, l'âge et la consommation d'alcool n'avaient pas d'effet significatif sur l'excrétion du 1-OHP chez l'humain. Van Rooij *et al.* (1994b) et Van Schooten *et al.* (1995) n'observèrent pas non plus d'effet de l'alcool sur l'excrétion du 1-OHP chez l'humain.

En résumé, étant donné que le pyrène est retrouvé en importante proportion dans les mélanges de HAP, que le 1-OHP est aisément mesurable dans l'urine humaine et que son excrétion est corrélée avec l'intensité de l'exposition, ce métabolite urinaire présente un intérêt particulier comme indicateur biologique de l'exposition aux HAP. L'influence de divers facteurs tels que la voie d'exposition, la fumée de cigarette, la nourriture riche en HAP, les facteurs exogènes (la période de l'année, le moment du prélèvement urinaire) et les facteurs d'hôtes (l'âge et le sexe) sur l'excrétion urinaire du 1-OHP est par ailleurs assez bien documentée autant chez l'animal que chez l'humain. En dépit d'une utilisation grandissante des mesures urinaires du 1-OHP dans la surveillance biologique de l'exposition

aux HAP, les données sur la toxicocinétique détaillée de ce métabolite sont toutefois incomplètes. Celles-ci permettraient cependant une meilleure compréhension des données de surveillance biologique provenant des études épidémiologiques où on n'effectue en général que des prélèvements urinaires ponctuels.

1.5. Autres données métaboliques pertinentes

1.5.1. Distribution et élimination hors de l'organisme

1.5.1.1. Distribution et élimination du BaP dans l'organisme

Les données sur la distribution du BaP dans l'organisme se résument essentiellement en une mesure du composé marqué dans les divers tissus et fluides biologiques chez l'animal (Bevan et Weyand, 1988; Foth et Kahl, 1988; Kotin *et al.*, 1959; Mitchell, 1982; Moody *et al.*, 1995; Weyand et Bevan, 1986; Withey *et al.*, 1993b). La distribution se fait initialement aux tissus adipeux, au foie et aux reins, peu importe la voie d'exposition. Le BaP est ensuite rapidement métabolisé en produits excrétés principalement dans la bile puis les fèces (Moody *et al.*, 1995; Withey *et al.*, 1993b). Seulement une faible quantité est excrétée dans l'urine. En effet, selon des expériences animales, 80 à 100% d'une dose de BaP sont éliminés par les fèces (Camus *et al.*, 1984; Sanders *et al.*, 1986; Van de Wiel *et al.*, 1993; Wolff *et al.*, 1989). Foth *et al.* (1988) et Weyand et Bevan (1986) rapportèrent que seulement 2% d'une dose de BaP marqué étaient récupérés dans l'urine. Il a par ailleurs été observé qu'une faible proportion de BaP marqué est retenue dans certains tissus, dont les tissus adipeux, pendant des semaines suivant une exposition (IARC, 1983; Lemaire *et al.*, 1990).

Suite à une administration du BaP marqué au ^{14}C ou ^3H , certains auteurs ont également quantifié différents métabolites dans ces divers compartiments après extraction et séparation par CLHP, collecte de fractions à partir du CLHP et comptage subséquent (Chipman *et al.*, 1981; Mollière *et al.*, 1987; Weyand et Bevan, 1986). Chipman *et al.* (1981) ont quantifié plusieurs métabolites du BaP dans la bile de rats exposés à 3 μmol de BaP- $^{14}\text{C}/\text{kg}$. La majorité des métabolites étaient des conjugués polaires et les métabolites majeurs retrouvés dans la bile après hydrolyse des glucuronoconjugués étaient le 4,5-diolBaP et le 3-OHBaP. Weyand et Bevan (1986) ont, pour leur part, déterminé les quantités de métabolites du BaP retrouvées dans les poumons, le foie et le système gastro-intestinal. Parmi ces métabolites, on comptait des diols, des quinones et des phénols.

Withey *et al.* (1993b) observèrent également que les concentrations de BaP et de ses métabolites (en mesurant la différence entre la radioactivité totale et le BaP mesuré spécifiquement par CLHP) étaient plus faibles dans le foie, les poumons et le sang six heures après inhalation de BaP- ^3H qu'immédiatement après exposition. Seuls les tissus graisseux possédaient des niveaux significativement plus élevés de BaP et de ses métabolites chez des rats sacrifiés six heures après exposition par rapport à ceux sacrifiés immédiatement après exposition. Ces auteurs suggérèrent que le BaP et ses métabolites se redistribuaient aux tissus graisseux durant les six heures suivant l'exposition.

1.5.1.2. Distribution et élimination du pyrène dans l'organisme

Withey *et al.* (1991; 1992; 1993a,b) ont étudié le comportement toxicocinétique du pyrène marqué au ^{14}C . La cinétique de distribution et d'élimination du ^{14}C total et du pyrène libre dans le sang et divers tissus a été déterminée chez des rats suite à une application cutanée de 2, 6 et 15 mg de pyrène- $^{14}\text{C}/\text{kg}$ (9,9, 29,7 et 74,2 $\mu\text{mol}/\text{kg}$) et des prélèvements sanguins et tissulaires un, deux, quatre et six jours après traitement (Withey *et al.*, 1993a). Les niveaux les plus élevés de ^{14}C total et de pyrène libre étaient observés dans les tissus adipeux, le foie et les reins. La demi-vie d'élimination du pyrène libre dans les divers tissus, calculée à partir des concentrations mesurées entre 24 et 144 heures après traitement, était de deux jours environ. La valeur de demi-vie d'élimination tissulaire du ^{14}C total était approximativement de quatre jours. Le pyrène- ^{14}C était rapidement excrété dans l'urine et les fèces et l'élimination était presque complète en quatre jours.

Withey *et al.* (1992) ont également étudié la cinétique de distribution sanguine et tissulaire du ^{14}C total et du pyrène libre suite à une exposition au pyrène- ^{14}C par inhalation (de 200 à 800 mg/m^3 (0,99 à 3,96 mmol/m^3) pendant 95 minutes) chez le rat et sacrifice zéro et six heures après exposition. Les concentrations de pyrène libre au temps zéro variaient comme suit: sang > poumons > tissus adipeux > foie > reins > coeur > fœtus et six heures après exposition: tissus adipeux > sang \approx reins > foie \approx poumons > coeur > fœtus. Par ailleurs, Withey *et al.* (1991) ont également examiné la distribution du ^{14}C total et du pyrène libre dans différents tissus suite à une exposition à 2, 4, 6, 9 et 15 mg de pyrène- $^{14}\text{C}/\text{kg}$ (9,9, 19,8, 29,7, 44,5 et 74,2 $\mu\text{mol}/\text{kg}$) par voies intraveineuse et orale. Les tissus n'ont cependant été prélevés qu'à un seul temps après exposition (de 5 à 15 heures après exposition selon

la dose). Les niveaux de ^{14}C total suivaient l'ordre: tissus adipeux > foie = reins = poumons > coeur = testicules = rate = cerveau. La cinétique du pyrène- ^{14}C et du pyrène libre dans le sang a par contre été tracée de façon plus détaillée. L'élimination était biphasique et des demi-vies de quatre et huit heures ont été calculées pour la phase terminale d'élimination du pyrène et du pyrène- ^{14}C , respectivement.

Dans les études mentionnées plus haut, la quantité de métabolites était considérée comme étant la différence entre le pyrène- ^{14}C total et le pyrène libre quantifié par CLHP. Ces expériences ne nous informent cependant pas, spécifiquement, sur le devenir du 1-OHP dans l'organisme. Bien que le 1-OHP soit le métabolite majeur du pyrène formé par des microsomes de foie de rats *in vitro* (Jacob *et al.*, 1982), d'autres métabolites sont également formés. Les données de ces derniers auteurs montrent que 57% des métabolites du pyrène quantifiés dans leur expérience correspondaient au 1-OHP alors que 43% représentaient d'autres métabolites soit le 4,5-dihydrodiol, le 1,6-dihydroxypyrene et un diphenol et un triol non identifiés.

Par ailleurs, il a été déterminé que le pyrène- ^{14}C total était excrété en proportions égales dans l'urine et les fèces suivant l'application cutanée de 2 et 6 mg de pyrène- $^{14}\text{C}/\text{kg}$ (9,9 et 29,7 $\mu\text{mol}/\text{kg}$) alors qu'un tiers de la quantité excrétée de ^{14}C total était éliminé dans l'urine et deux tiers dans les fèces suivant une application cutanée de 15 mg de pyrène- $^{14}\text{C}/\text{kg}$ (74,2 $\mu\text{mol}/\text{kg}$) (Withey *et al.*, 1993a). Suivant une exposition par inhalation, Withey *et al.* (1994) rapportèrent que 53% de la quantité totale de pyrène- ^{14}C excrétée étaient récupérés dans l'urine pendant une période de collectes de six jours par rapport à 47% dans les fèces et ce, indépendamment de la dose. Après exposition par voie orale

ou intraveineuse, 45 et 40% de la dose de pyrène-¹⁴C administrée étaient excrétés dans l'urine et les fèces, respectivement, pendant une période de collectes de six jours (Withey *et al.*, 1991). Or, en analysant spécifiquement, par CLHP, le 1-OHP excrété dans l'urine de rats, il a été indiqué plus haut que moins de 1% d'une dose aiguë orale, intraveineuse ou cutanée était excrété sous forme de 1-OHP en 48 heures et qu'il n'y avait plus d'excrétion appréciable de ce métabolite par la suite (Dodd et Viau, non publié). Jacob *et al.* (1989) ont également rapporté que seulement un très faible pourcentage de la dose de pyrène était excrété sous forme de 1-OHP dans l'urine, soit 0,4% après administrations intrapéritonéale et orale, 0,3% après instillation intratrachéale et moins de 0,01% après instillation intrapulmonaire.

Ces divergences apparentes démontrent la nécessité d'examiner, suite à une administration du pyrène marqué, autant la distribution et l'élimination de la radioactivité totale que du pyrène et 1-OHP spécifiquement. Encore une fois, une utilisation adéquate du 1-OHP dans l'urine comme bioindicateur de la dose absorbée de pyrène, et donc de HAP dans une situation d'exposition humaine environnementale ou en milieu de travail, ne sera possible que lorsque la relation entre l'exposition au composé parent et l'élimination du 1-OHP sera clairement établie.

1.5.2. Considérations sur le transport biliaire

Comme mentionné précédemment, les HAP sont éliminés en grande partie dans la bile puis dans les fèces (Bevan et Weyand, 1988; Chipman *et al.*, 1981; Foth et Kahl, 1988; Weyand et Bevan, 1986; Wolff *et al.*, 1989). Le transport biliaire des anions est un transport actif, donc saturable (Sathirakul

et al., 1994). Une telle saturation pourrait par conséquent mener à une élimination urinaire accrue. Dans le cas du 1-OHP, étant donné le faible pourcentage excrété dans l'urine (<1% chez le rat), même une diminution infime de l'excrétion biliaire (par exemple 1%) pourrait avoir une implication importante sur l'augmentation des niveaux d'excrétion urinaire. Une telle saturation pour le transport biliaire de l'ensemble des métabolites du BaP a déjà été démontrée par Kotin *et al.* (1959), chez des rats, à partir d'une dose de 3,2 μmol de BaP- $^{14}\text{C}/\text{kg}$. Ces auteurs rapportèrent une plus faible récupération (% de la dose injectée) du BaP- ^{14}C dans la bile après administration d'une dose de 400 μg (1,6 μmol) par rapport à une dose de 26, 45 et 134 μg du produit marqué (0,1, 0,18 et 0,53 μmol). Withey *et al.* (1991) ont de plus rapporté que, chez des rats, 37% et 12% de la dose étaient excrétés via la bile sur une période de six heures après administration de 2 mg de pyrène- $^{14}\text{C}/\text{kg}$ (9,9 $\mu\text{mol}/\text{kg}$) par voies intraveineuse et orale, respectivement, alors qu'en augmentant la dose à 9 mg/kg (44,5 $\mu\text{mol}/\text{kg}$), le pourcentage excrété dans la bile chutait à 27% et 8% pour les mêmes voies.

Il est par ailleurs intéressant de noter que le poids moléculaire et la polarité influencent l'excrétion biliaire d'un composé (Klaassen et Watkins, 1994; Smith, 1973). La biotransformation, notamment la conjugaison, augmente l'excrétion biliaire en introduisant des centres polaires forts dans la molécule et en augmentant le poids moléculaire. Par exemple, le 1-OHP est majoritairement excrété sous forme de glucurono-conjugué dans les fèces chez le rat (Boyland et Sims, 1964). Ce conjugué possède une masse moléculaire de 378, une valeur quelque peu supérieure à celle qui est normalement considérée comme la valeur seuil de l'excrétion biliaire, valeur en dessous de laquelle peu d'élimination hépatique se produit, soit 325 chez le rat (Klaassen et Rozman, 1991; Klaassen et Watkins, 1991; Smith, 1973). Cette valeur seuil serait plus élevée, soit de l'ordre de 500, chez l'humain (Klaassen et

Watkins, 1994), ce qui devrait causer une moindre excrétion biliaire chez celui-ci. Ces observations semblent compatibles avec les données recueillies par notre groupe sur l'excrétion urinaire de 1-OHP chez l'humain et chez le rat. En effet, suivant une administration par voie orale, l'humain excrète quelque dix fois plus de pyrène sous forme de 1-OHP que le rat (3,7% contre 0,36%). Soulignons cependant qu'il existe de nombreuses exceptions aux valeurs seuils de transport biliaire selon les substances (Klaassen et Watkins, 1994).

Il ressort donc, entre autres, que l'effet de la dose sur l'excrétion urinaire et biliaire du 1-OHP mérite d'être étudié d'autant plus que les données à ce sujet se rapportent essentiellement à une étude chez la truite dans laquelle une seule dose avait été administrée (Law *et al.*, 1994).

En terminant, en plus d'être un processus saturable, le transport biliaire est susceptible d'être sujet à de l'inhibition compétitive (Sathirakul *et al.*, 1994). Puisque la plupart des HAP formeront des métabolites mono-hydroxylés, une inhibition compétitive du transport biliaire des conjugués du 1-OHP, due par exemple à la présence d'autres conjugués d'autres HAP, est plausible. Cette inhibition entraînerait une plus grande concentration plasmatique des conjugués 1-OHP et donc une plus grande élimination par voie urinaire de ces derniers pour une même dose d'exposition au pyrène.

1.5.3. La problématique des mélanges de HAP

Étant donné que les HAP sont présents sous forme de mélanges, on peut s'interroger sur l'influence possible de l'exposition à plusieurs HAP sur l'excrétion urinaire des métabolites d'un HAP spécifique. Comme mentionné précédemment, les HAP subissent l'action d'un groupe d'enzymes appelées les mono-oxygénases à fonctions multiples, celles-ci regroupant les isoenzymes du cytochrome P450. Conney *et al.* (1994) ont observé que le BaP, le 3-méthylcholanthrène et le 7,12-diméthylbenz(a)anthracène (DMBA) induisaient l'activité BaP hydroxylase, c'est-à-dire le système enzymatique métabolisant le BaP en produits phénoliques fluorescents alors que le pyrène et l'anthracène n'avaient aucun effet sur son activité. Ceci indique que la présence de certains HAP dans un mélange peut conduire à un effet sur le métabolisme d'autres membres de cette même famille de composés dans le mélange. Un tel effet a également été observé par Jacob *et al.* (1982) dans des études *in vitro* impliquant des microsomes de foies de rats. Dans cette étude, il a été démontré que le BaP induisait le métabolisme du pyrène.

Les études ou expériences portant sur les mélanges de HAP et l'excrétion urinaire de métabolites de HAP spécifiques se limitent en général à des expositions à la créosote ou au goudron de houille chez l'animal ou l'humain. Ainsi, par exemple, Jongeneelen *et al.* (1986), en exposant des rats par voie cutanée au goudron de houille, montrèrent que l'excrétion urinaire du 3-OHBaP et 1-OHP augmentait de façon proportionnelle à la dose suggérant que les quantités excrétées de ces deux métabolites n'étaient pas influencées par la présence d'autres HAP contenus dans le mélange. Par ailleurs, certains auteurs ont étudié l'excrétion urinaire du 1-OHP chez des patients psoriasiques

suivant une application cutanée de goudron de houille pur ou d'onguents à base de goudron de houille et observèrent une augmentation du 1-OHP suivant le traitement (Clonfero *et al.*, 1989; Hansen *et al.*, 1993b; Jongeneelen *et al.*, 1985a) et tout au long de la période de traitement (Jongeneelen *et al.*, 1986). L'influence d'un HAP du mélange sur la biotransformation des autres membres du groupe ou encore l'effet de l'administration du mélange par rapport à l'administration des composés individuels sur les niveaux d'excrétion urinaire des métabolites ne firent pas partie de ces études. Ces études ne permettent donc pas de comprendre les mécanismes d'interactions entre les membres du mélange ou même de mettre en évidence des interactions puisqu'elles n'ont pas été réalisées en conjonction avec des études sur les composés individuels ou sur des mélanges plus simples.

Récemment, comme indiqué plus haut, la cinétique d'excrétion urinaire détaillée du 1-OHP a été établie chez des volontaires suivant une application cutanée de créosote et chez un patient psoriasique suivant un traitement avec un shampoing à base de goudron de houille (Viau et Vyskocil, 1995). En comparant le profil cinétique et la valeur de demi-vie d'élimination obtenus chez ces volontaires exposés à la créosote et chez ce patient psoriasique par rapport à des volontaires exposés au pyrène seul (Viau *et al.*, 1995a), il ressort que le profil global d'excrétion du 1-OHP n'est pas influencé par une exposition concomitante à d'autres HAP aux concentrations contenues dans la créosote ou dans le shampoing à base de goudron de houille. Il reste toutefois à s'interroger sur l'importance quantitative de cette excrétion par rapport à l'excrétion obtenue avec le pyrène pur et à examiner les données en fonction de la composition particulière des mélanges utilisés.

1.6. Problématique du projet de recherche

1.6.1. Problématique générale

On exprime un intérêt grandissant pour le développement et la validation de bioindicateurs de l'exposition aux HAP dans des études épidémiologiques et dans l'analyse du risque encouru par l'exposition à ces contaminants environnementaux ubiquistes. D'une part, étant donné l'importance quantitative du pyrène dans la plupart des sources d'émissions et étant donné la facilité de mesurer le 1-OHP, le métabolite majeur du pyrène dans l'urine animale et humaine, ce métabolite a été suggéré comme bioindicateur de l'exposition globale aux HAP. Néanmoins, les données sur la toxicocinétique détaillée de ce bioindicateur sont relativement pauvres. L'utilisation adéquate de celui-ci dans le contexte de l'épidémiologie moléculaire où des collectes ponctuelles sont effectuées chez des individus ne peut se faire en l'absence de ces données sur la toxicocinétique fondamentale.

D'autre part, le BaP présente un intérêt dans les études de toxicocinétique sur les HAP étant donné son potentiel cancérigène. Les métabolites urinaires du BaP, bien que facilement mesurables chez l'animal, ne sont toutefois pas quantifiés aisément chez l'humain avec les méthodes analytiques courantes. Il demeure tout de même important de connaître le comportement cinétique de ces derniers chez l'animal, celui-ci pouvant être comparé avec celui du 1-OHP. Par ailleurs, il reste que la mise au point de méthodes plus sensibles que celles utilisées à ce jour pourrait permettre la détection aisée des métabolites urinaires du BaP chez l'humain.

1.6.2. Problématiques spécifiques

1.6.2.1. Variation de l'excrétion urinaire des métabolites du BaP selon la dose, la voie d'administration, l'espèce, la souche et le sexe

Il existe des différences importantes dans les résultats obtenus par différents chercheurs concernant l'excrétion urinaire des métabolites du BaP chez l'animal. Celles-ci résultent entre autres de variations dans l'excrétion de ces métabolites selon la dose, la voie d'exposition, l'espèce, la souche et le sexe. Bien que plusieurs auteurs aient étudié l'effet de la dose, de la voie d'exposition ou du moment d'échantillonnage sur l'excrétion urinaire de plusieurs métabolites spécifiques du BaP, aucune étude systématique visant à examiner ces différents facteurs simultanément n'a été rapportée. Il apparaît dès lors important d'incorporer ces divers paramètres dans un seul modèle expérimental et sous les mêmes conditions. Par ailleurs, étant donné que plusieurs métabolites du BaP sont connus avec des cinétiques d'excrétion probablement différentes et puisqu'un métabolite donné peut être excrété en quantités différentes selon la voie d'exposition, une mesure des ratios de concentrations de ces métabolites pourrait apporter de l'information sur le moment et la voie majeure d'exposition. Ce concept présenterait un grand intérêt pour l'évaluation de l'exposition en milieu de travail et permettrait de mieux comprendre comment les travailleurs sont exposés et, en conséquence, comment mieux adapter les mesures de protection.

1.6.2.2. Limite des données concernant la cinétique détaillée de l'excrétion urinaire du 1-OHP et des métabolites du BaP

Il existe des failles dans nos connaissances sur la cinétique d'excrétion urinaire du 1-OHP qui doivent être comblées afin d'établir les conditions appropriées pour l'utilisation du bioindicateur. De plus, mis à part les résultats d'une expérience publiée récemment par notre groupe sur le 3-OHBaP, les cinétiques détaillées d'excrétion urinaire de métabolites du BaP n'ont pas été étudiées et étant donné, comme indiqué plus haut, que le 1-OHP est utilisé comme bioindicateur de l'exposition globale aux HAP, il apparaît intéressant de comparer le comportement cinétique du 1-OHP dans l'urine avec celui des métabolites du BaP. Par ailleurs, des études antérieures ont suggéré au moins deux phases d'élimination dans la cinétique détaillée d'excrétion urinaire du 3-OHBaP et du 1-OHP. Il semble donc également intéressant d'examiner si la biotransformation est une étape limitante dans la cinétique d'excrétion du 1-OHP et des métabolites du BaP après administration du composé parent.

1.6.2.3. Insuffisance des données se rapportant à la balance entre l'excrétion urinaire et biliaire du 1-OHP

Chez l'humain, on se réfère à une mesure du 1-OHP dans l'urine comme bioindicateur de l'exposition. Or, des expériences animales ont montré qu'une importante proportion de la dose de pyrène administrée semblait être éliminée par voie biliaire et fécale. Le transport biliaire est toutefois un processus saturable et sujet à de l'inhibition compétitive. Toute saturation ou inhibition de celui-ci pourrait mener à une redistribution du trop plein vers la voie urinaire. Il est donc essentiel de fixer

l'importance relative de l'excrétion biliaire et urinaire du 1-OHP en particulier en fonction de la dose et de la voie d'administration.

1.6.2.4. Absence de données quantitatives sur l'influence de l'exposition à des mélanges de HAP sur l'excrétion urinaire du 1-OHP

Étant donné qu'en milieu de travail, nous sommes exposés à des mélanges de HAP et non pas à des HAP individuels, il est important de déterminer si l'exposition aux mélanges aurait un effet sur la cinétique d'excrétion urinaire et sur la quantité excrétée du 1-OHP et s'il existe une dose seuil en dessous de laquelle cet effet hypothétique ne serait pas observé.

1.6.2.5. Pauvreté des données concernant la cinétique de distribution et d'élimination détaillée du pyrène et du 1-OHP dans l'organisme

Bien que la cinétique de distribution et d'élimination du pyrène marqué et du pyrène libre soit assez bien documentée, le comportement cinétique des métabolites spécifiques dans l'organisme est moins bien connue. Dans les études publiées dans la littérature sur la toxicocinétique du pyrène chez l'animal, la quantité de métabolites dans le sang et les tissus a été estimée comme étant la différence entre la mesure de radioactivité totale et la détermination spécifique du pyrène inchangé. Néanmoins, suite à l'exposition au pyrène, des différences importantes ont été obtenues dans les quantités récupérées de métabolites dans l'urine et les fèces, selon que l'on estime la quantité totale de métabolites à partir des mesures de la radioactivité totale et du pyrène libre ou que l'on détermine le

1-OHP spécifiquement. Il est donc important de mieux comprendre la relation entre l'exposition au composé parent et l'élimination du 1-OHP afin de permettre une meilleure interprétation des résultats de la surveillance biologique appliquée à l'analyse du risque.

1.7. Objectifs de recherche

1.7.1. Objectif général

L'objectif général de ce projet de recherche sera donc de parfaire nos connaissances sur la cinétique détaillée de l'excrétion urinaire du 1-OHP selon l'importance des doses administrées, selon les voies d'exposition et selon que l'exposition se fait au pyrène seul ou à des mélanges simples de HAP. La cinétique d'excrétion urinaire détaillée des métabolites du BaP sera également étudiée et comparée à celle du 1-OHP. Il s'agira de plus de mieux documenter la cinétique de distribution et d'élimination du pyrène et du 1-OHP dans l'organisme. Cette démarche permettra une meilleure interprétation des données de surveillance biologique de l'exposition aux HAP et donc une meilleure évaluation du risque posé par l'exposition à ces contaminants environnementaux considérés comme polluants prioritaires au Canada. Étant donné que le rat présente des profils d'excrétion urinaire du 1-OHP semblables à ceux qui sont observés chez des volontaires humains, ce modèle animal s'avère adéquat pour permettre l'acquisition de certaines données sur la toxicocinétique du 1-OHP et des métabolites du BaP qui ne peuvent être obtenues chez des volontaires humains pour des raisons d'éthique.

1.7.2. Objectifs spécifiques

- 1) Examiner l'effet de la voie d'administration, de la dose et du moment d'échantillonnage sur l'excrétion urinaire des métabolites du BaP (Article 1).
- 2) Documenter la cinétique détaillée de l'excrétion urinaire des métabolites du BaP et du 1-OHP suite à l'administration du BaP et du pyrène, respectivement, ou après traitement avec les métabolites eux-mêmes pour examiner l'importance de la biotransformation sur la cinétique urinaire des métabolites du BaP et du 1-OHP après administration du composé parent (Article 2).
- 3) Étudier l'effet de la voie d'administration et de la dose de pyrène administrée sur l'excrétion du 1-OHP par voies urinaire et biliaire (Article 3).
- 4) Étudier l'effet de l'exposition à des mélanges binaires et ternaires de HAP sur la cinétique détaillée de l'excrétion urinaire du 1-OHP (Article 4).
- 5) Déterminer la cinétique de distribution et d'élimination de la radioactivité totale, du pyrène libre et, plus particulièrement, du 1-OHP dans l'organisme de rats exposés à une dose aiguë de pyrène-¹⁴C par voie intraveineuse (Article 5).

CHAPITRE DEUXIÈME

PRÉSENTATION DES ARTICLES

ARTICLE 1

Urinary Excretion of Benzo(a)pyrene Metabolites following Intravenous, Oral and Cutaneous Benzo(a)pyrene Administration

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Abstract

The effect of the administration route, dose and sampling time on the total urinary excretion of four major benzo(a)pyrene (BaP) metabolites, 3-hydroxyBaP (3-OHBaP), 9-hydroxyBaP (9-OHBaP), trans-4,5-dihydrodiolBaP (4,5-diolBaP) and trans-9,10-dihydrodiolBaP (9,10-diolBaP), was studied in male Sprague-Dawley rats exposed to a single intravenous, oral and cutaneous dose of 2, 6, 20 and 60 $\mu\text{mol BaP/kg}$. Urine samples were collected at 24-h intervals following treatment. Over the 0-72 h period and for a given dose, amounts of BaP metabolites were 3-OHBaP > 4,5-diolBaP >> 9-OHBaP following intravenous and oral dosing, and 3-OHBaP >> 9-OHBaP \geq 4,5-diolBaP after cutaneous treatment. 9,10-diolBaP was barely detected. On the other hand, amounts of 3-OHBaP and 4,5-diolBaP excreted in urine over the 0-72 h period and for a given dose appeared in the following order: intravenous \approx oral \geq cutaneous. Amounts of 9-OHBaP excreted varied as follows: oral \geq cutaneous > intravenous. For all routes of administration, excretion of 4,5-diolBaP was almost complete over the 0-24 h period in contrast with 3-OHBaP and 9-OHBaP. Peak excretion of 3-OHBaP and 9-OHBaP was reached in the 0-24 h period following intravenous and oral treatment and in the 24-48 h period following cutaneous application. Overall, for a given administration route and dose, there were variations in the time profiles between metabolites. In general, there was nonetheless a good correlation between the BaP dose and urinary excretion of 3-OHBaP, 9-OHBaP and 4,5-diolBaP. Furthermore, total urinary excretion of a specific metabolite, its time profile, and the relative proportion of the metabolites studied depended on the administration route. Data also suggest that a measure of the concentration ratio of the different metabolites could reflect the time and main route of exposure.

Key words: benzo(a)pyrene, urinary metabolites, route of administration, rats.

Résumé

L'effet de la voie d'administration, de la dose et du moment d'échantillonnage sur l'excrétion urinaire de quatre métabolites majeurs du benzo(a)pyrène (BaP), le 3-hydroxyBaP (3-OHBaP), le 9-hydroxyBaP (9-OHBaP), le trans-4,5-dihydrodiolBaP (4,5-diolBaP) et le trans-9,10-dihydrodiolBaP (9,10-diolBaP), a été étudié chez des rats mâles Sprague-Dawley exposés à une dose aiguë de 2, 6, 20 ou 60 μmol de BaP/kg par voies intraveineuse, orale et cutanée. Trois collectes urinaires consécutives de 24 h ont été effectuées. Les résultats montrent que, pour une dose donnée et pour la période 0-72 h, les quantités relatives des métabolites excrétés étaient: 3-OHBaP > 4,5-diolBaP >> 9-OHBaP suivant administration par voies intraveineuse et orale et 3-OHBaP >> 9-OHBaP \geq 4,5-diolBaP après application cutanée. Le 9,10-diolBaP était à peine décelable. Par ailleurs, pour une dose donnée, les quantités de 3-OHBaP et de 4,5-diolBaP excrétées pendant la période 0-72 h variaient selon la voie d'administration comme suit: intraveineuse \approx orale \geq cutanée. Celles du 9-OHBaP suivaient plutôt l'ordre: orale \geq cutanée > intraveineuse. Peu importe la voie d'administration, l'excrétion du 4,5-diolBaP était presque complète en 24 h contrairement à celle du 3-OHBaP et du 9-OHBaP. Le pic d'excrétion du 3-OHBaP et du 9-OHBaP était observé dans la période 0-24 h suivant l'administration intraveineuse et orale et dans la période 24-48 h suivant l'application cutanée. Donc, pour une voie d'entrée et dose données, d'un métabolite à l'autre, des différences dans la vitesse d'élimination ont été observées. En général, une bonne relation dose-excrétion était toutefois observée. En fonction de la voie d'administration, des variations dans l'excrétion totale et dans le profil temporel d'un métabolite urinaire spécifique ainsi que dans les proportions relatives des métabolites quantifiés ont aussi été notées. Les données suggèrent qu'une mesure des ratios de concentrations des métabolites pourrait fournir des indications sur le moment

et la voie majeure d'exposition.

Mots clés: benzo(a)pyrène, métabolites urinaires, voie d'administration, rats.

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous environmental contaminants, some of which have been classified as probable human carcinogens by the International Agency for Research on Cancer (IARC, 1987). Exposure to PAHs in the general environment can result from inhalation of ambient air, from ingestion of contaminated food, and from exposure to cigarette smoke (Lioy et al. 1988; Zhao et al. 1992). Highest exposures are however encountered in workers of coke industries and processes in which carbonaceous materials such as coke, coal tar pitch and asphalt oils are produced or used (Bjorseth et al. 1981; Lindstedt and Sollenberg, 1982). Exposure in occupational environments occurs mainly through the lungs and skin (van Rooij, 1994; ACGIH, 1991; IARC, 1984). To carefully control exposure to these substances, urinary excretion products have been employed as biomarkers (Ariese et al. 1994; Jongeneelen, 1987).

Among the various PAHs, benzo(a)pyrene (BaP) is one of the most studied. Toxicokinetics of BaP urinary metabolites in experimental models have been the focus of several research projects (Likhachev et al. 1992; van de Wiel et al. 1993; Jongeneelen et al. 1984). Specific metabolites of BaP have been quantified in urine and these include mainly 3-hydroxyBaP (3-OHBaP), trans-4,5-dihydrodiolBaP (4,5-diolBaP), trans-7,8-dihydrodiolBaP (7,8-diolBaP), 9-hydroxyBaP (9-OHBaP)

Abbreviations: BaP, benzo(a)pyrene; 3-OHBaP, 3-hydroxybenzo(a)pyrene; 9-OHBaP, 9-hydroxybenzo(a)pyrene; 4,5-diolBaP, trans-4,5-dihydrodiolbenzo(a)pyrene; 9,10-diolBaP, trans-9,10-dihydrodiolbenzo(a)pyrene; 7,8-diolBaP, trans-7,8-dihydrodiolbenzo(a)pyrene; PAH, polycyclic aromatic hydrocarbon; HPLC, high performance liquid chromatography; MeOH, methanol.

and trans-9,10-dihydrodiolBaP (9,10-diolBaP) (Bouchard and Viau, 1995; Tyndyk et al. 1994; van de Wiel et al. 1993; Likhachev et al. 1992; Jongeneelen et al. 1984; Camus et al. 1984). Studies looking at BaP metabolites in urine have also established the influence of the route of administration on the quantitative yield of specific metabolites (van de Wiel et al. 1993; Jongeneelen et al. 1984), examined relative importance of urinary and faecal excretion (van de Wiel et al. 1993; Likhachev et al. 1992) and attempted to identify biomarkers of susceptibility to the carcinogenic effect of BaP (Tyndyk et al. 1994; Likhachev et al. 1992).

Jongeneelen et al. (1984) have compared the urinary excretion of 3-OHBaP in Wistar rats following percutaneous penetration and oral absorption of repeated BaP doses. Amounts of 3-OHBaP excreted in urine and its excretion profile was found to vary with the administration route and, following percutaneous penetration, the percentage of BaP dose recovered as 3-OHBaP in urine depended on the administered dose. Likhachev et al. (1992) and Tyndyk et al. (1994) have examined the excretion of two BaP metabolites, 3-OHBaP and 7,8-diolBaP, in LIO rats and monkeys following single or repeated intraperitoneal BaP injection. They reported significant individual and species variations in the excretion of these metabolites. Camus et al. (1984) have looked at the excretion of three BaP metabolites, 3-OHBaP, 4,5-diolBaP and 7,8-diolBaP, in two mice strains, following a single intraperitoneal BaP dose. They showed strain differences in the molar fraction of the dose excreted as metabolites and in the relative proportion of the metabolites recovered in urine. Finally, Van de Wiel et al. (1993) have determined the effect of a large BaP dose on the urinary excretion of five major BaP metabolites, 3-OHBaP, 9-OHBaP, 4,5-diolBaP, 7,8-diolBaP and 9,10-diolBaP, following oral and intraperitoneal BaP dosing in male and female Wistar rats. It was determined that for a given

administration route, molar fraction of the dose recovered as phenolic metabolites in urine was greater in male than female rats. It also appeared that the amounts of phenolic metabolites excreted in urine and hence the metabolite quantitative profile depended on the administration route.

Although these studies were valuable contributions, none offered the opportunity to critically examine, within the same experimental framework, the relationship between quantitative urinary excretion of several BaP metabolites and route of administration, dose or time of sampling. Apparent species, strain and sex differences in the quantitative excretion of BaP metabolites in urine further restrains comparison of data between the various published studies. On the other hand, for the purpose of exposure assessment based on biological monitoring results, it is useful to reconstruct the exposure scenario from the determination of a metabolite, or series of metabolites, measured in a spot urine sample. The comparative study of the various metabolite kinetic profiles could then shed light on the time of exposure and could also provide insights as to the main route of entry of the PAH (e.g. ratio of the various metabolites could change with route of exposure and time since exposure). The objective was therefore to compare the total excretion of four major urinary metabolites of BaP, 3-OHBaP, 9-OHBaP, 4,5-diolBaP and 9,10-diolBaP in three consecutive 24-h urine samples following an acute intravenous, oral and cutaneous BaP administration in a single experimental animal, namely male Sprague-Dawley rats.

Materials and methods

Chemicals

BaP (approximately 98% purity) was purchased from Sigma (St Louis, Mo) and used as received. BaP metabolite reference standards (>99% purity) were obtained from the NCI Chemical Carcinogen Reference Standards distributed by Midwest Research Institute (Kansas, Mo) and used as received. β -glucuronidase/arylsulfatase (100 000 Fishman U/mL and 800 000 Roy U/mL from *Helix pomatia*) was purchased from Boehringer Mannheim GmbH (West-Germany). Alkamuls EL-620 (formerly emulphor 620) was obtained from Rhone-Poulenc (Canada). HPLC grade methanol (MeOH) was obtained from Caledon (Georgetown, ON, Canada). Anthracene and ascorbic acid were purchased from Baker Analyzed Biochemical (J.T. Baker Chem. Co, Phillipsburg, NJ).

Animals

Male Sprague-Dawley rats (Charles River Canada Inc., St-Constant, Quebec) of approximately 200 g were used. The animals were kept in individual metabolic cages cleaned daily and were given food and tap-water *ad libitum*. Lighting was maintained on a 12-h light-dark cycle and room temperature was kept at 22 ± 3 °C. Animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Experimental protocol

Animal treatment

Rats received a single dose of 2, 6, 20 and 60 $\mu\text{mol BaP/kg}$ ($n=4$ per dose) by intravenous injection, gavage (i.e. gastric intubation) and cutaneous application. For intravenous dosing, BaP was administered in a tail vein after being dissolved in 20% emulphor: 80% isotonic aqueous glucose solution (54.06 g glucose/L) until a clear solution was obtained. One millilitre of this solution was injected per kg body weight for the 2, 6, and 20 $\mu\text{mol BaP/kg}$ dose or 3 mL/kg for the 60 $\mu\text{mol/kg}$ dose. For the highest doses, solutions were slightly heated (approximately 37°C) and stirred continuously during filling of the syringes to maintain a clear solution. Visual examination of the animals ensured that physical condition was not altered by treatment. For oral administration, BaP was dissolved in corn oil and 1 mL of this solution was administered per kg body weight. BaP was dissolved in toluene for cutaneous application and 400 $\mu\text{L/kg}$ was applied to $\sim 6\text{ cm}^2$ portion of shaved dorsal skin just below the neck to prevent rats from licking or rubbing the application site.

Urine sampling

Twenty-four-hour urine samples were collected during the 72 h following treatment (i.e. urine samples 0-24 h, 24-48 h, and 48-72 h post-dosing). Control 24-h urine samples were collected prior to dosing.

Pretreatment of urine for HPLC analysis

Current methods for the biological monitoring of exposure to PAHs using urinary metabolites involve the determination of the latter on hydrolysed urine samples (Jongeneelen and Anzion, 1991; Jongeneelen et al. 1987). Therefore, in the present report, total metabolites were measured, these being the sum of unconjugated and conjugated forms. Urine samples were collected over thymol and kept frozen at -20°C until analysis. Urine samples were treated using the method of Jongeneelen et al. (1984). Briefly, urine samples were adjusted to pH 5 with 4.0 N HCl and buffered with 5 mL of 0.1 M sodium acetate (pH 5). This solution was incubated overnight (16 h) with 12.5 μL β -glucuronidase (1250 U)/arylsulfatase (10 000 U) in a shaking bath at 37°C . Samples were centrifuged to eliminate cellular debris and other residues. Extraction of the metabolites was performed by passing the samples through sep-pak C-18 cartridges (Waters, Millipore, Milford, Ma). Cartridges were primed with 4 mL of HPLC grade MeOH and 8 mL of milliQ water. Samples were then passed through the cartridges, washed with 8 mL of milliQ water and the retained solutes were eluted with 8 mL of MeOH. The solvent was evaporated at 50°C under nitrogen flow. The residue was dissolved in 1.5 mL of MeOH containing 1 mg/L of anthracene which was used as an external standard. Efficiency of the extraction of hydrolysed metabolites was determined using untreated rat urine samples spiked with 12.5, 25, 50 pmol or 100 pmol of authentic reference standards and analysed as previously described. Recovery from at least 25 spiked samples was $17 \pm 1\%$ (mean percentage \pm SE) for 3-OHBaP, $27 \pm 2\%$ for 9-OHBaP, $80 \pm 1\%$ for 4,5-diolBaP and $83 \pm 3\%$ for 9,10-diolBaP. Results were therefore corrected for recovery. In other tests with both concentrated and diluted urine using a large range of BaP metabolite enrichment, we have identified that the major steps where losses occurred were centrifugation after enzymatic hydrolysis and extraction on Sep-Paks (Bouchard

and Viau, 1996). However, during this extensive testing, we have observed that the percentage recovery was remarkably constant whatever the dilution of urine or the level of enrichment.

A preliminary experiment was also conducted to establish optimal conditions for hydrolysis of the sulfo- and glucurono-conjugates of the four metabolites studied. Quadruplicate samples of pooled and diluted urine from rats treated with an acute intraperitoneal dose of 400 μmol BaP/kg were incubated with 12.5 μL β -glucuronidase/arylsulfatase, in a shaking bath at 37 $^{\circ}\text{C}$, for periods of 2, 4, 6, 8, 16, 24 or 48 h, and analysed as previously described. No statistically significant differences were observed between the mean values obtained for the different periods of hydrolysis (data not shown).

HPLC analysis

The system consisted of a model AS-100 HPLC automatic sampling system (Bio-Rad, Richmond, CA), a model 250 binary pump (Perkin-Elmer, Buckingham, England), a 150 \times 4.6 mm Supelco LC 18 column (Supelco, Oakville, ON, Canada) and a Perkin-Elmer LS-40 fluorescence detector. Analysis was performed in 70 min using a methanol:water mobile phase and elution conditions described by Van de Wiel et al. (1993). Therefore, the following solvent program was used: 5 min 90% solvent A (60% water:40% HPLC grade methanol), a linear gradient to 90 % solvent B (100% methanol) in 40 min, followed by 10 min 90% solvent B. Program returned to initial conditions using a linear gradient to 90% solvent A in 10 min, and 5 min 90% solvent A to re-equilibrate the column. Ascorbic acid (1mg/L) was added to the eluents to improve analytical conditions (Bouchard et al. 1994). The injection volume was 20 μL throughout. Column temperature was 40 $^{\circ}\text{C}$ and flow 1

mL/min. The program of excitation and emission wavelengths was that described by Van de Wiel et al. (1993). Excitation and emission wavelengths were first set to 280 and 406 nm, respectively, for optimal detection of 9,10-diolBaP. After 21 and 31 min, these wavelength pairs were set to 265/405 nm and 265/430 nm for optimal detection of 4,5-diolBaP and of 9-OHBaP and 3-OHBaP, respectively. The detector signal was recorded and treated with PE Nelson turbochrom 3 software. Retention times of 9,10-diolBaP, 4,5-diolBaP, anthracene, 9-OHBaP and 3-OHBaP were 18.5, 28, 37, 39 and 40 (± 0.3) min, respectively. Random methanolic extracts of treated rat urine samples were spiked with a mixture of the four BaP metabolite reference standards to ascertain peak identity. Detection limit was 0.8 to 3 nmol/L, depending on the metabolite. Intra-day coefficient of variations (C.V.) of positive controls, which correspond to aliquots of pooled urine from rats treated with BaP, were 6 % for 3-OHBaP, 11% for 9-OHBaP, 5 % for 4,5-diolBaP and 11% for 9,10-diolBaP (n=4). Inter-day C.V. were 10 % for 3-OHBaP, 18% for 9-OHBaP, 10 % for 4,5-diolBaP and 13% for 9,10-diolBaP (n=5 analysed over 24 days). Concentration range of the standard curve was 6.25 - 50 nmol/L of methanol. Coefficient of variations of standard curve slopes calculated over a period of 21 days were 5 % for 3-OHBaP, 7 % for 9-OHBaP, 0.9 % for 4,5-diolBaP and 0.7 % for 9,10-diolBaP.

Statistical analysis

Values are presented as arithmetic means \pm standard error of the mean (SE). One-way analysis of variance (ANOVA) was used to test overall differences, with respect to BaP dose, in the molar fraction of dose excreted as BaP metabolites considering a given metabolite and a given administration route. Bonferroni post-hoc test was performed between all pairs of dose groups again

considering a given metabolite and a given administration route; $p=0.05$ was considered as the level of significance.

Results

Table 1 shows the total urinary excretion of 3-OHBaP, 9-OHBaP and 4,5-diolBaP over the 0-72-h period post-dosing. In Table 2, values are expressed as the mean percentage of the BaP dose excreted as the various metabolites that were measured. For a given dose, relative amounts of metabolites were as follows: 3-OHBaP > 4,5-diolBaP >> 9-OHBaP following intravenous and oral dosing, and 3-OHBaP >> 9-OHBaP ≥ 4,5-diolBaP after cutaneous application. For a given dose, the amounts of 3-OHBaP and 4,5-diolBaP excreted in urine during the 0-72 h period after treatment occurred in the following order: intravenous ≈ oral > cutaneous. The amount of 9-OHBaP excreted varied as follows: oral ≥ cutaneous > intravenous. For all administration routes, a good correlation between the BaP dose and the urinary excretion of 3-OHBaP, 9-OHBaP, and 4,5-diolBaP was observed. Hence, the dose had no significant effect on the percentage of metabolites excreted except for 4,5-diolBaP after oral dosing.

Table 3 shows the effect of exposure route, dose and sampling time on the quantitative urinary excretion of 3-OHBaP, 9-OHBaP and 4,5-diolBaP. For a given exposure route and dose, time profiles differed according to the metabolite. For all administration routes, 3-OHBaP was excreted in greater amounts than 4,5-diolBaP. Almost all 4,5-diolBaP was excreted during the 0-24 h period and was, thereafter, barely detectable in contrast with 3-OHBaP. On the other hand, although 3-OHBaP was excreted in greater amounts than 9-OHBaP for all exposure routes, similar time profiles were obtained for these two metabolites. Also, the amounts excreted and time profiles of a given metabolite varied as a function of the route of administration. Comparable time profiles and amounts

excreted for the different sampling periods were obtained for 3-OHBaP, following intravenous and oral dosing, and peak values were reached in the 0-24 h period. On the contrary, 3-OHBaP peak excretion was observed in the 24-48 h period following cutaneous application. The effect of the administration route on the time profile of 9-OHBaP was comparable to what was observed for 3-OHBaP. However, for all administration routes, 4,5-diolBaP peak excretion was reached in the 0-24 h period following dosing although, as mentioned previously, for a given dose, amounts excreted were dependent on the exposure route.

The excretion of 9,10-diolBaP was also studied. However, the results were not included in this report because peak appeared in the early portion of the chromatogram and was often hardly distinguishable from other fluorescent materials in urine. Excretion values also often approached limit of detection. The quality of the information was therefore insufficient.

Table 1. Urinary excretion of 3-OHBaP, 9-OHBaP and 4,5-diolBaP in the 0-72 h period following intravenous, oral and cutaneous administration of 2, 6, 20 and 60 $\mu\text{mol BaP/kg}$ in male Sprague-Dawley rats

BaP metabolite excretion mean \pm SE ¹ (pmol per 72 h)	Dose ($\mu\text{mol/kg}$)	Administration route		
		Intravenous	Oral	Cutaneous
3-OHBaP	2	261 \pm 74	292 \pm 53	263 \pm 77
	6	1179 \pm 92	1185 \pm 378	529 \pm 167
	20	1999 \pm 431	2832 \pm 401	1525 \pm 556
	60	8514 \pm 2773	7234 \pm 2503	2439 \pm 504
9-OHBaP	2	ND ²	12 \pm 7	ND
	6	11 \pm 11	41 \pm 14	58 \pm 21
	20	24 \pm 19	239 \pm 57	81 \pm 23
	60	164 \pm 40	357 \pm 100	278 \pm 65
4,5-diolBaP	2	147 \pm 44	28 \pm 8	12 \pm 4
	6	690 \pm 282	251 \pm 62	16 \pm 3
	20	950 \pm 182	1287 \pm 275	51 \pm 13
	60	3797 \pm 1385	3682 \pm 798	187 \pm 53

¹ n=4 per dose

² ND= non detectable

Table 2. Mean percentage of the BaP intravenous, oral and cutaneous dose excreted in urine as 3-OHBaP, 9-OHBaP and 4,5-diolBaP over the 0-72 h period following administration[†]

Urinary metabolite (mean percentage of the BaP dose \pm SE ¹)	Dose (μ mol/kg)	Administration route		
		Intravenous	Oral	Cutaneous
3-OHBaP	2	0.058 \pm 0.016	0.062 \pm 0.012	0.055 \pm 0.016
	6	0.085 \pm 0.007	0.083 \pm 0.027	0.038 \pm 0.012
	20	0.043 \pm 0.009	0.058 \pm 0.009	0.032 \pm 0.011
	60	0.061 \pm 0.020	0.051 \pm 0.018	0.018 \pm 0.004
9-OHBaP	2	ND ²	0.0026 \pm 0.0016	ND
	6	0.0008 \pm 0.0008	0.0029 \pm 0.0010	0.0042 \pm 0.0015
	20	0.0005 \pm 0.0004	0.0050 \pm 0.0012	0.0018 \pm 0.0005
	60	0.0012 \pm 0.0003	0.0025 \pm 0.0007	0.0020 \pm 0.0005
4,5-diolBaP	2	0.032 \pm 0.009	0.006 \pm 0.002*	0.0026 \pm 0.0009
	6	0.050 \pm 0.020	0.018 \pm 0.004	0.0012 \pm 0.0002
	20	0.020 \pm 0.004	0.026 \pm 0.006	0.0011 \pm 0.0003
	60	0.027 \pm 0.010	0.026 \pm 0.006	0.0013 \pm 0.0004

¹ n= 4 per dose

² ND= non detectable

[†] Statistical analyses were performed to evaluate if, considering a given metabolite and a given administration route, the difference among the dose group means was significant

* Statistically significant difference among the dose group means. However, when comparing pairs of dose groups, post-hoc Bonferroni p values were not significant

Table 3. Excretion of 3-OHBaP, 9-OHBaP and 4,5-diolBaP in three consecutive 24-h urine samples of male Sprague-

Dawley rats treated with 2, 6, 20 and 60 $\mu\text{mol BaP/kg}$ by the intravenous, oral and cutaneous routes

BaP metabolite excretion mean \pm SE ¹ (pmol per day)	Dose ($\mu\text{mol/kg}$)	Administration route								
		Intravenous			Oral			Cutaneous		
		0-24 h	24-48 h	48-72 h	0-24 h	24-48 h	48-72 h	0-24 h	24-48 h	48-72 h
3-OHBaP	2	222 \pm 69	39 \pm 7	ND ²	237 \pm 56	55 \pm 8	ND	94 \pm 37	137 \pm 37	32 \pm 23
	6	965 \pm 76	170 \pm 35	43 \pm 8	1052 \pm 363	133 \pm 18	ND	162 \pm 60	284 \pm 104	83 \pm 41
	20	1683 \pm 369	229 \pm 70	86 \pm 46	2620 \pm 390	189 \pm 19	24 \pm 14	574 \pm 161	688 \pm 472	264 \pm 150
	60	7025 \pm 2552	1055 \pm 145	433 \pm 154	5528 \pm 1970	1627 \pm 644	78 \pm 22	966 \pm 102	1269 \pm 470	204 \pm 75
9-OHBaP	2	ND	ND	ND	12 \pm 7	ND	ND	ND	ND	ND
	6	11 \pm 11	ND	ND	41 \pm 14	ND	ND	19 \pm 9	39 \pm 16	ND
	20	16 \pm 9	8 \pm 8	ND	227 \pm 59	12 \pm 7	ND	32 \pm 8	49 \pm 18	ND
	60	134 \pm 38	30 \pm 2	ND	309 \pm 73	47 \pm 31	ND	85 \pm 37	192 \pm 34	ND
4,5-diolBaP	2	147 \pm 44	ND	ND	28 \pm 8	ND	ND	12 \pm 4	ND	ND
	6	690 \pm 282	ND	ND	251 \pm 62	ND	ND	16 \pm 3	ND	ND
	20	950 \pm 185	ND	ND	1287 \pm 275	ND	ND	51 \pm 13	ND	ND
	60	3695 \pm 1426	102 \pm 102	ND	3628 \pm 804	54 \pm 32	ND	187 \pm 53	ND	ND

¹ n= 4 per dose

² ND= non detectable

Discussion

This study has been undertaken to examine the effect of exposure route, dose and sampling time on the urinary excretion of BaP metabolites to better assess the usefulness of these metabolites in urine as biomarkers of BaP exposure. Since BaP yields several urinary metabolites, it is particularly well suited as a model compound for the study of the potential advantage of measuring the relative excretion of these metabolites to better reconstruct the exposure scenario. It is well known that a large proportion (up to virtually 100%) of the BaP dose is eliminated in the bile and hence in the faeces (Camus et al. 1984; Sanders et al. 1986; Weyand and Bevan, 1986; Wolff et al. 1989). However, in the context on biological monitoring, the urinary route is of interest. Furthermore, although with the method developed by Jongeneelen et al. (1984) BaP metabolites in urine are not easily detectable in occupationally exposed individuals, they are measured in psoriatic patients which are exposed to high doses of PAH during treatment with ointments or shampoos containing coal tar (Jongeneelen et al. 1986). Ariese et al. (1994) have moreover developed a very sensitive method than enables the measurements of major hydroxylated BaP metabolites, namely 3-OHBaP, in the urine of exposed workers and controls. Concentrations of 3-OHBaP as low as 0.5 ng/L have been detected. On the other hand, while 1-OHP presently appears as a better bioindicator of exposure to PAHs, it remains important to know the toxicokinetic behaviour of other PAH metabolites in urine such as BaP metabolites for comparison with 1-OHP urinary excretion kinetics.

The present study showed that total amounts of a specific metabolite excreted in urine over the 0-72 h period following treatment were largely influenced by the administration route. In general, total

amounts of 3-OHBaP or 4,5-diolBaP excreted were comparable after intravenous injection and gavage and values were higher than after cutaneous application. Meanwhile, levels of 9-OHBaP were, in general, similar or higher after oral administration than after cutaneous application and intravenous injection. Van de Wiel et al. (1993) also reported differences in the amounts of excreted products after oral or intraperitoneal BaP dosing in male and female rats. Total amounts of metabolites excreted in urine were almost 4 times higher after intraperitoneal dosing than after oral administration in male rats whereas these values were 3 times smaller, following intraperitoneal injection, than oral dosing in female rats.

When expressing excretion values as percentage of the BaP dose recovered in urine as the various metabolites, it was calculated that 0.043 to 0.085 % of the intravenous dose (mean value for a given dose), 0.051 to 0.083 % of the oral dose, and 0.018 to 0.055 % of the cutaneous dose was excreted as 3-OHBaP. These values obtained in the current report were somewhat comparable to the mean value of 0.015 % that can be estimated from results of Likhachev et al. (1992) after an acute intraperitoneal injection of 200 mg BaP/kg in LIO rats but lower than the values of 0.22 to 0.35 % reported by Jongeneelen et al. (1985) after repeated oral dosing in Wistar rats where a probable enzymatic induction occurred. On the other hand, in a previously published study in rats exposed intravenously to 40 μ mol BaP/kg, it was observed that, on the average, 0.1% of the dose was recovered in urine as 3-OHBaP (Bouchard and Viau, 1996).

Furthermore, for a given dose, time profiles of specific metabolites appeared to vary according to the administration route. 3-OHBaP and 9-OHBaP excretion peaked within the first 24 h following oral

and intravenous treatment whereas peak values were reached in the 24-48 h period following cutaneous application. However, comparable time profiles of 4,5-diolBaP urinary excretion were observed for all administration routes. Van de Wiel et al. (1993) also observed differences in the excretion time profiles, depending on the exposure route. Maximum excretion of total metabolites was reached on days 2 and 3 following intraperitoneal dosing, and on day one following oral exposure.

Similar behaviour of each of the two major BaP metabolites in urine, namely 3-OHBaP and 4,5-diolBaP, following intravenous and oral administration suggests that oral absorption is extremely effective, that the manner in which BaP distributes to the various tissues is similar by these two routes and that the major site of biotransformation is the same. It has been shown that several tissues such as the liver, lungs, kidneys and skin can biotransform PAHs (Bronaugh et al. 1994; Ueng and Alvares, 1993; Mollière et al. 1987). Owing to the first-pass extraction of the chemical by the liver following oral administration, it appears that biotransformation occurs mainly in the liver following administration by these two routes. Roth and Vinegar (1990) have also indicated a significant contribution of the lungs to the systemically available BaP. The similarities in BaP excretion pattern following intravenous and oral administration could also be explained by the fact that the enzymatic system implicated in the biotransformation of BaP by the two routes are in the same proportions. On the other hand, discrepancies in the absolute and relative urinary excretion of BaP metabolites following administration by the cutaneous route as compared to the intravenous and oral routes suggest that the major biotransformation site is different and in the former case would be the skin. Indeed, tissue variations in the expression (types and amounts) of the relevant enzymes have been

reported (Zedeck, 1980) and Bronaugh et al. (1994) indicated that aryl hydrocarbon hydroxylase activity in liver is more than 10 times that of skin. Kao et al. (1985) have also shown that the metabolic viability was a major factor involved in the in vitro skin permeation of applied BaP and that absorption was conditioned by metabolic processes. According to Ng et al. (1992), absorption of BaP through the skin is mediated by biotransformation processes. Alternatively, delay in peak excretion after cutaneous application could also be explained by a reservoir effect of the dermis (Chu et al. 1996).

On the other hand, following all administration routes, a linear dose-dependent excretion of 3-OHBP, 9-OHBP and 4,5-diolBP was observed which suggests that BaP metabolizing enzymes were not saturated over the range of doses used in the present study. These findings also render unlikely that only the BaP in intimate contact with the stratum corneum is bioavailable. There were no statistically significant differences in the percentage of the dose excreted as BaP metabolites over the range of doses administered except the effect of oral dose on the urinary excretion of 4,5-diolBP. No clear explanation can be given to this last observation although urinary excretion levels were closer to the detection limit after oral administration of the 2 μmol BaP/kg dose. Jongeneelen et al. (1984; 1985) noted a good relationship between dose and urinary excretion of 3-OHBP following repeated oral BaP treatment. In contrast, Sanders et al. (1986) reported that following percutaneous ^{14}C -labelled BaP treatment in mice, the percentage of the dose absorbed, as measured by the amount of radioactivity recovered in the tissues and excreta, decreased with increasing dose. Indeed, it has been shown that both diffusional and metabolic processes are involved in the percutaneous absorption

of BaP (Ng et al. 1992; Kao et al. 1985). Ng et al. (1992) investigated the *in vitro* skin absorption of BaP and showed that the amount of metabolite that permeated the skin into a receptor fluid was the same for the low and high dose groups suggesting that enzymatic activity can be saturated at high doses. Jongeneelen et al. (1984) in fact reported that in rats treated percutaneously with 10, 20 and 50 μmol BaP/kg on three consecutive days, the percentage of the dose recovered in urine as 3-OHBaP was highest at the lowest dose applied. In the current report, it was also observed that the percentage of the BaP cutaneous dose excreted as 3-OHBaP tended to decrease with increasing dose suggesting a possible saturation in the uptake process or in the skin metabolism at the highest doses. However, considering a given metabolite and a given administration route, overall differences between the dose group means were not statistically significant.

Effect of the vehicle on the cutaneous absorption of BaP should also be considered. Since application of pure BaP was impossible, a lipophilic solvent that evaporated very rapidly was chosen to dissolve BaP in the current report. With the range of doses tested, toluene appeared as a better solvent than acetone. Of course, toluene could defat the skin and facilitate transport through the stratum corneum. Alternatively, a certain percentage of oil could have been added to the solvent. This would nonetheless have increased the penetration of BaP by acting as a vehicle for the transport BaP through the skin. A certain retention in the oil could also have occurred.

With the administration route and dose taken into consideration, variations in the time profiles between metabolites in urine were also observed in the present study. For all administration routes, elimination of 4,5-diolBaP was almost complete within 24 h post-dosing whereas 3-OHBaP and 9-

OHBaP were more persistent in urine. These findings agree with those of a previously published study where the detailed kinetic profiles of 3-OHBaP and 4,5-diolBaP were established following intravenous BaP injection (Bouchard and Viau, 1996). Indeed, excretion kinetics of 3-OHBaP showed at least two steps, a first step persisting over 24 h and a second step having a first order elimination half-life of 8.1 h. In contrast, elimination of 4,5-diolBaP was linear and a first order apparent half-life of 3.1 h was calculated.

It should be kept in mind that the hydrolysed urine samples contain the conjugated and unconjugated forms of a metabolite and that differences in excretion profiles exist between these two forms. Although some authors have reported that the conjugated metabolites had longer urinary elimination rates, were excreted later or exhibited elimination half-lives longer than the unconjugated metabolites or the parent compounds (Woodworth et al. 1986; Schänzer et al. 1991; Prelusky et al. 1986), it is generally accepted that conjugated compounds are more polar and more readily excreted than their precursors (deBethizy and Hayes, 1994; Barnhart and Combes, 1978; Smith and Williams, 1966). This would therefore suggest that 4,5-diolBaP is probably excreted mainly in the conjugated form since excretion was complete in 24 h whereas total 3-OHBaP and 9-OHBaP are excreted as both conjugated and unconjugated species since total metabolites were still excreted between 24 and 72 h post-dosing.

In addition, differences in the time profiles and excretion rates of 3-OHBaP, 9-OHBaP and 4,5-diolBaP suggest a probable relationship between the concentration ratio of these metabolites and exposure pattern. For example, knowing the route of exposure, a measure of the concentration ratio

3-OHBP/4,5-diolBP could reveal the time of exposure. Indeed, considering an exposure mainly through the diet, a low ratio value would indicate a more recent exposure whereas a high ratio value would mean a more remote exposure. Furthermore, for a given dose, the urinary time profiles of specific BP metabolites appeared to vary according to exposure route, and for a given urine sampling period and dose, the relative proportion of different BP metabolites excreted in urine also varied with administration route. Knowing the time of exposure, study of the concentration ratio of different metabolites could therefore determine the importance of exposure route. For example, a urine sample collected shortly after an exposure with a high 3-OHBP/4,5-diolBP ratio could suggest an exposure mainly by the cutaneous route. While these metabolites are presently not measured routinely in urine of occupationally exposed workers since extremely sensitive methods are required, this concept presents a great interest for evaluating exposure in a work environment, perhaps providing a useful tool to better understand how workers are exposed and, consequently, how to adapt better protection measures.

In conclusion, there were variations in the concentration ratios of the various BP metabolites quantified for a given exposure route and dose in the 0-72 h period after dosing. Differences in the time profiles according to the metabolite were also apparent. Furthermore, the administration route had an effect on the amounts excreted and time profile of a specific metabolite. A measure of the concentration ratios of various urinary BP metabolites could therefore indicate the time and main route of exposure. This report also shows that a good linear dose-excretion relationship was generally observed over a large range of doses.

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ARTICLE 2

Urinary Excretion Kinetics of Pyrene and Benzo(a)pyrene Metabolites following Intravenous Administration of the Parent Compounds or the Metabolites

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ABSTRACT

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The detailed urinary excretion profiles of 1-hydroxypyrene (1-OHP) and benzo(a)pyrene (BaP) metabolites were studied following acute intravenous administration of pyrene and BaP, respectively, or after injection of the metabolites themselves. Male Sprague-Dawley rats were exposed to 4 μmol 1-OHP/kg or 15 μmol pyrene/kg. Other rats were exposed to 2 μmol /kg of a mixture of four BaP metabolites (3-hydroxyBaP (3-OHBaP), 9-hydroxyBaP (9-OHBaP), trans-4,5-dihydrodiolBaP (4,5-diolBaP) and trans-9,10-dihydrodiol (9,10-diolBaP)) or 40 μmol BaP/kg. Urine samples were collected at frequent intervals over 48 or 96 hr. Injection of both pyrene and 1-OHP produced similar biphasic excretion profiles. An apparent first order half-life of 6.9 and 6.6 hr, respectively, could be calculated for the second phase of elimination. Comparable 3-OHBaP excretion profiles were obtained after injection of BaP or a mixture of BaP metabolites. Elimination kinetics showed at least two steps, the second step having a first order apparent half-life of 8.1 and 7.6 hr following BaP and BaP metabolites injection, respectively. Time profiles of 4,5-diolBaP excretion following administration of BaP or a mixture of BaP metabolites were almost identical. Elimination was linear and a first order apparent half-life of 3.1 and 3.6 hr could be calculated. Elimination of 4,5-diolBaP was much more rapid than that of 3-OHBaP and complete within 24 hr post-dosing. Therefore, results suggest that 1) phase I biotransformation is not the rate-limiting step in the excretion of 1-

OHP, and 3-OHBaP and 4,5-diolBaP following injection of pyrene and BaP, respectively, and 2) similarities in the first order apparent half-life of 3-OHBaP and 1-OHP for the late phase of excretion suggest that 1-OHP could be a good surrogate for 3-OHBaP.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs), including the probable human carcinogen benzo(a)pyrene (BaP), have been listed as Priority Substances to be assessed under the Canadian Environmental Protection Act (Meek *et al.*, 1994). It is therefore important to thoroughly monitor exposure to these substances in the frame of risk assessment. A priori, it would appear interesting to look at BaP metabolites in urine as bioindicators of exposure to total PAHs. However, although several BaP urinary metabolites are easily detectable in rodents (Camus *et al.*, 1984; van de Wiel *et al.*, 1993; Likhachev *et al.*, 1992), 3-hydroxyBaP (3-OHBaP), a major BaP urinary metabolite in animals, has only exceptionally been measured at trace levels in coke oven workers (Ariese *et al.*, 1994) and small concentrations were measured in psoriatic patients exposed to a high dose of BaP through application of a coal tar ointment (Jongeneelen *et al.*, 1986).

In contrast, since 1-hydroxypyrene (1-OHP), the major urinary metabolite of pyrene (Boyland and Sims, 1964; Keimig *et al.*, 1983), is easily detectable in humans including the general population (Viau *et al.*, 1995b) and since pyrene represents an important proportion of most PAH mixtures (Buchet *et al.*, 1992; Roussel *et al.*, 1992), more and more studies resort to this urinary metabolite as a bioindicator of exposure to total PAHs. Even though 1-OHP appeared to be a useful bioindicator of exposure to PAHs (Levin, 1995), few data are available on its detailed toxicokinetics which is essential for a proper interpretation of a measure of 1-OHP in spot urine samples of exposed individuals, in the context of epidemiological studies .

Several researchers have looked at the crude excretion kinetics of 1-OHP in workers. Pre- and post-shift spot urine samples were collected over the workweek (Jongeneelen *et al.*, 1985; van Rooij *et al.*, 1993; Jongeneelen *et al.*, 1988), or, daily or twice-a-day samples were collected during the weekends (Jongeneelen *et al.*, 1985; Jongeneelen *et al.*, 1988; Buchet *et al.*, 1992), or during a prolonged holiday (Jongeneelen *et al.*, 1988). Jongeneelen *et al.* (1988) observed two elimination phases in the excretion kinetics of 1-OHP in workers with half-lives of 1 to 2 days, and 16 days, respectively. The first phase would correspond to the rapidly available portion of the body burden (i.e. the central compartment and tissues in direct equilibrium) while the second phase would correspond to a slower component presumably the elimination of pyrene accumulated in slowly perfused tissues such as adipose tissues. These results were however based on a small number of samples and individuals allowing only a crude determination of the kinetic parameters. In a cohort of creosote workers, Viau *et al.* (1993) observed that even after 2 weeks without exposure in the workplace, the subjects under study still excreted 1-OHP in concentrations significantly higher than unexposed controls, suggesting the possible existence of a long-term component in people having an important body burden of parent PAHs or of the metabolites.

Later, Jongeneelen *et al.* (1990) estimated a half-life of 6 to 35 hr for the initial phase of elimination in 18 coke oven workers. Boogaard and van Sittert (1994) estimated a half-life of 13.4 hr in workers of a petrochemical industry. Although a large number of individuals were used, data were based on a small number of urine samples. Buchet *et al.* (1992) reported, however, a half-life of 18 hr for the first phase of elimination described by Jongeneelen *et al.* (1988) using more frequent urine samples and a large number of individuals. Furthermore, in a semi-controlled study where 1-OHP was

administered through diet, a half-life of 4.4 hr was estimated from urine samples collected at intervals of 8 h (Buckley and Lioy, 1992). Kang *et al.* (1995) also examined the urinary excretion levels of 1-OHP-glucuronide in volunteers samples (first morning voiding) collected before (days -2, 1), during (days 2-5) and after (days 6-8,15) beef consumption. A 10 to 80-fold increase in 1-OHP-glucuronide concentrations was observed on day 2 of beef consumption and concentrations approached baseline values by 24-72 h after the end of the feeding period.

Recently, more detailed excretion profiles of urinary 1-OHP (at intervals of 0.5 to 4.4 hr) were determined in volunteers exposed to acute doses of pyrene by the oral (Viau *et al.*, 1995a) and dermal (Viau *et al.*, 1995a; Viau and Vyskocil, 1995) routes. At least two phases of elimination were observed and a first order apparent half-life of approximately 12 hr was obtained for both routes of exposure for the 12 to 48 hr phase. The second phase described by Jongeneelen *et al.* (1988) was not observed.

Additionally, other researchers have looked at the crude excretion kinetics of 1-OHP (Jongeneelen *et al.*, 1985; Jacob *et al.*, 1989) or 3-OHBaP (Jongeneelen *et al.*, 1984; Jongeneelen *et al.*, 1986; Likhachev *et al.*, 1992) in rodents. However, 24-hr urine samples were collected which were insufficient to allow for a proper determination of the kinetic parameters. Recently, we have determined the detailed excretion kinetics of 1-OHP following administration of pyrene by various routes. The kinetic profiles were similar to those observed in volunteers (Viau *et al.*, 1995a; Viau and Vyskocil, 1995). First order apparent half-life of 6.3, 7.0 and 8.6 hr were calculated for the 12 to 48 hr phase following oral, intravenous and cutaneous treatments, respectively (Dodd and Viau,

unpublished). The similarities in the kinetic behaviors in rats and humans suggest that the rat is a suitable model for studies on the toxicokinetics of urinary 1-OHP.

Furthermore, to our knowledge, the detailed excretion kinetics of BaP urinary metabolites after administration of BaP have not yet been established and would be useful for comparison with 1-OHP urinary excretion kinetics. It also appears interesting to look at the importance of phase I biotransformation on the toxicokinetics of 1-OHP and BaP metabolites after administration of the parent compound. Therefore, the detailed urinary excretion profiles of 1-OHP and BaP metabolites (3-OHBaP, 9-hydroxyBaP (9-OHBaP), trans-4,5-dihydrodiolBaP (4,5-diolBaP) and trans-9,10-dihydrodiol (9,10-diolBaP)) were determined following intravenous administration of the parent compounds, pyrene or BaP, or the metabolites themselves.

MATERIALS AND METHODS

Chemicals. Pyrene was purchased from Baker Analyzed Biochemical (J.T. Baker Chem. Co, Phillipsburg, NJ) and used as received. BaP (approximately 98% purity) was purchased from Sigma (St-Louis, MO) and used as received. 1-OHP and BaP metabolites reference standards (>99% purity) were obtained from the NCI Chemical Carcinogen Reference Standards distributed by Midwest Research Institute (Kansas, MO) and used as received. Alkamuls EL-620 (formerly emulphor 620) was obtained from Rhone-Poulenc (Canada). HPLC grade MeOH was obtained from Caledon (Georgetown, ON, Canada). Anthracene, naphthalene and ascorbic acid were purchased from Baker Analyzed Biochemical (J.T. Baker Chem. Co, Phillipsburg, NJ).

Animals. Male Sprague-Dawley rats (Charles River Canada Inc., St-Constant, Quebec) of 200 to 250 g were used. The animals were kept in individual metabolic cages cleaned daily. Lighting was maintained on a 12 hr light-dark cycle and room temperature was kept at 22 ± 3 °C.

Animal treatment. Rats received a single intravenous dose of 4 μmol 1-OHP/kg or 15 μmol pyrene/kg (n=5 per group) by a tail vein. Other rats received a single intravenous dose of 2 $\mu\text{mol}/\text{kg}$ of a mixture of BaP metabolites (3-OHBaP, 4,5-diolBaP, 9-OHBaP and 9,10-diolBaP) or 40 μmol BaP/kg (n=5 per group). Vehicle used for injection was a 20% emulphor: 80% isotonic glucose solution. For the administration of 1-OHP and the mixture of BaP metabolites, 3 ml of solution was injected per kg of body weight. For pyrene and BaP administration, 4 ml of this solution was injected per kg of body weight.

Urine sampling. For 1-OHP analysis, all micturitions were collected at frequent intervals over a 48-hr period. Urine samples were collected every 2 hr for the first 12 hr post-dosing; this was made possible by adding D-glucose (40 g/l) and saccharin (1.5 g/l) to the drinking water to induce a polydipsic behavior with associated aqueous diuresis (Chouinard and Viau, 1992). Collection of samples was made every 6 hr for the following 18 hr, and during 6 and 12 hr for the last 18 hr. Control urine samples were collected from the same animals during the 4 hr preceding injection.

For BaP metabolites analysis, micturitions were again collected at frequent intervals but for up to 96 hr post-dosing. Urine samples were collected every 2 hr for the first 12 hr post-dosing (see above), every 6 hr for the following 18 hr, and alternately every 6 and 12 hr for the last 66 hr. Control 2-hr urine samples were collected 0 to 2 hr, and 2 to 4 hr prior to dosing.

Urine analysis. Urine samples were collected over thymol and frozen at -20°C until analysis. Urine samples were treated using the method of Jongeneelen *et al.* (1987). Efficiency of the extraction of hydrolyzed metabolites was determined using untreated rat urine samples spiked with 12.5, 25, 50, 100 pmol of authentic reference standards. Recovery from at least 16 spiked samples was $65 \pm 2\%$ (mean percentage \pm SE) for 1-OHP, $50 \pm 3\%$ for 3-OHBaP, $43 \pm 2\%$ for 9-OHBaP, $79 \pm 2\%$ for 9,10-diolBaP and $84 \pm 2\%$ for 4,5-diolBaP. Samples collected in the 12 to 48-hr period following injection of pyrene or 1-OHP, or in the period 24 to 96-hr following BaP or BaP metabolites administration, were more concentrated than the samples collected during the initial aqueous diuresis period. Later samples were therefore centrifuged to facilitate passage through Sep-paks. As a result, however, recovery from at least 25 spiked samples dropped to $47 \pm 1\%$ (mean percentage \pm SE) for

1-OHP, $17 \pm 1\%$ for 3-OHBaP and $27 \pm 2\%$ for 9-OHBaP, and was $83 \pm 3\%$ for 9,10-diolBaP and $80 \pm 1\%$ for 4,5-diolBaP. Results presented in this article were adjusted for recovery.

Analysis was performed using a High Performance Liquid Chromatographic (HPLC) system consisting of a model AS-100 HPLC automatic sampling system (Bio-Rad, Richmond, CA), a model 250 binary pump (Perkin-Elmer, Buckingham, England), and a Perkin-Elmer LS-40 fluorescence detector. The detector signal was recorded and treated with PE Nelson Turbochrom 3 software.

For 1-OHP analysis, a LC-18 Supelcosil 250×4.6 mm column (Supelco, Oakville, ON, Canada) was used. Analysis was performed in 25 min using a 71:29 methanol:water mobile phase. Recovery and reproducibility of the chromatography was improved by the addition of 1 mg/l of ascorbic acid to the methanol eluent (Bouchard *et al.*, 1994). The injection volume was 20 μ l throughout. Column temperature was 32°C and flow 0.8 ml/min. Excitation and emission wavelengths were 242 and 388 nm respectively. Concentration range of the standard curve was 6.25 - 50 nmol/l of methanol. Naphthalene (1 mg/l) was used as an external standard.

For BaP metabolites analysis, a 150×4.6 mm Supelco LC 18 column (Supelco, Oakville, ON, Canada) was used. The injection volume was 20 μ l throughout. Column temperature was 40°C and flow 1 ml/min. Analysis was performed in 70 min with elution conditions described by Van de Wiel *et al.* (1993). Ascorbic acid (1 mg/l) was added to the eluent to improve analytical conditions (Bouchard *et al.*, 1994). Anthracene (1 mg/l) was used as an external standard. Excitation and emission wavelengths were first set to 274 and 404 nm, respectively, for the optimal detection of

9,10-diolBaP. After 21 and 31 min, these wavelength pairs were set to 265/383 nm and 265/430 nm for the optimal detection of 4,5-diolBaP and of 9-OHBaP and 3-OHBaP, respectively. Concentration range of the standard curve was 6.25 - 50 nmol/l of methanol.

RESULTS

Urinary excretion kinetics of 1-OHP following intravenous injection of pyrene or 1-OHP

Figure 1 shows similar time profiles of 1-OHP urinary excretion after injection of pyrene or the metabolite itself. Excretion kinetics showed at least two elimination phases, and a first order apparent half-life of 6.9 and 6.6 hr could be estimated for the 12 to 42 hr phase following intravenous injection of pyrene and 1-OHP, respectively. Excretion levels of 1-OHP had reached background levels in most rats for the last sampling period between 42 and 48 hr post-dosing. Furthermore, over 98% of the pyrene and 1-OHP dose excreted as 1-OHP in urine over the 48-hr collection period was eliminated during the first 24 hr post-dosing.

Urinary excretion of 3-OHBaP following intravenous injection of BaP or a mixture of BaP metabolites

Figure 2 shows comparable urinary excretion profiles of 3-OHBaP following intravenous administration of BaP or the mixture of four BaP metabolites. Maximum excretion rates were observed only 10 hr post-dosing for both exposures. Elimination appeared linear after 24 h post-dosing and slopes were parallel for both excretion curves. A first order apparent half-life of 8.1 and 7.6 hr could be calculated for the 24 to 66 hr phase following administration of BaP and BaP

metabolites, respectively.

On the average, 63 and 95 % of the dose excreted as 3-OHBaP was eliminated during the first 24 and 48 hr, respectively, following administration of BaP which was comparable to the values of 69 and 97 % obtained after BaP metabolites administration. It is also noteworthy that 3-OHBaP was non detectable in all samples obtained prior to dosing.

Urinary excretion of 4,5-diolBaP following intravenous injection of BaP or a mixture of BaP metabolites

Figure 3 shows the time profiles of 4,5-diolBaP urinary excretion following administration of BaP or the mixture of BaP metabolites. Excretion kinetics were almost identical when injecting the parent compound or the urinary metabolites. Maximum excretion rates were reached in the first 2-hr sampling period post-dosing. Elimination appeared linear for the 2 to 30 hr phase following administration of BaP or BaP metabolites and a first order apparent half-life of 3.1 and 3.6 hr, respectively, was calculated. Furthermore, excretion was rapid and almost complete within 24 hr post-dosing. Indeed, virtually 100% of the total amount measured over the 96-hr collection period was eliminated within the first 24 hr.

Urinary excretion of 9-OHBaP and 9,10-diolBaP following intravenous injection of BaP or a mixture of BaP urinary metabolites

Urinary excretion of 9-OHBP and 9,10-diolBP was also followed over a 96-hr collection period but excretion values were very small, when detectable, and number of data was insufficient to compare the excretion kinetics following administration of BP or BP urinary metabolites. Nonetheless, 9-OHBP was detectable in the first urine collection samples but was not detectable in most samples within 6 to 8 hr post-dosing. Peak of 9,10-diolBP was only detectable in the 0- to 2-hr urine collection period following dosing when injecting BP or BP metabolites. Unfortunately, 9,10-diolBP analysis was complicated by the fact that excretion values were small and unidentified fluorescent materials in urine interfered somewhat with 9,10-diolBP peak.

Half-life of urinary 1-OHP and BP metabolites following administration of pyrene or 1-OHP and following injection of BP or a mixture of four metabolites of BP

Table 1 summarizes the values of half-life for 1-OHP following administration of pyrene or 1-OHP as well as the half-lives for 3-OHBP and 4,5-diolBP after intravenous injection of BP or the mixture of metabolites. Both 1-OHP and 3-OHBP excretion kinetics showed at least two steps, and comparable first order apparent half-lives were obtained for 1-OHP and 3-OHBP for the second step of elimination. However, the half-lives of these metabolites were not determined for the same period of time following dosing.

Furthermore, it was observed that 4,5-diolBP was eliminated much faster than 3-OHBP. Values of 4,5-diolBP peaked immediately following injection whereas peak values for 3-OHBP were only observed after 10 hr post-dosing. Half-life determined for 4,5-diolBP was about half that of 3-

OHBaP even though they were not calculated over the same period of time following administration. Figure 4 shows the decrease in the ratio of 4,5-diolBaP/3-OHBaP urinary excretion with time since injection of BaP or the metabolites.

Determination of the mean percentage of the dose excreted as urinary metabolites

Table 2 shows that both 1-OHP and BaP metabolites were excreted in much greater amounts after administration of the metabolites themselves than after injection of the parent compounds. Molar fraction of the 1-OHP dose excreted as urinary 1-OHP was 15 times that of pyrene dose excreted as 1-OHP. Molar fraction of the 3-OHBaP dose excreted as 3-OHBaP in urine was 7 times that of BaP dose excreted as 3-OHBaP. Molar fraction of the dose excreted in urine as 4,5-diolBaP following administration of a mixture of BaP metabolites was 34 times higher than that obtained after BaP dosing.

It is also noteworthy that 9-OHBaP was excreted in greater amounts than 9,10-diolBaP. The mean percentage of the BaP dose excreted as 9-OHBaP was 45 and 50 times less than that excreted as 4,5-diolBaP and 3-OHBaP, respectively. In addition, the fraction of the BaP metabolites dose excreted followed the order 4,5-diolBaP > 3-OHBaP > 9-OHBaP. After injection of BaP, fraction of BaP dose excreted was as follows: 3-OHBaP = 4,5-diolBaP > 9-OHBaP.

Finally, molar fraction of the pyrene dose excreted as 1-OHP in urine was 7 times that of BaP dose excreted as 3-OHBaP whereas the mean percentage of 1-OHP dose excreted as urinary 1-OHP was

15 times that of BaP metabolites dose excreted as 3-OHBaP. Furthermore, when looking at the ratio of 1-OHP or BaP metabolites excreted following administration of the parent compound over those excreted following administration of the metabolites, value obtained for 1-OHP was half that observed for 3-OHBaP. Ratio obtained for 3-OHBaP was 3 and 5 times higher than those calculated for 9-OHBaP and 4,5-diolBaP, respectively.

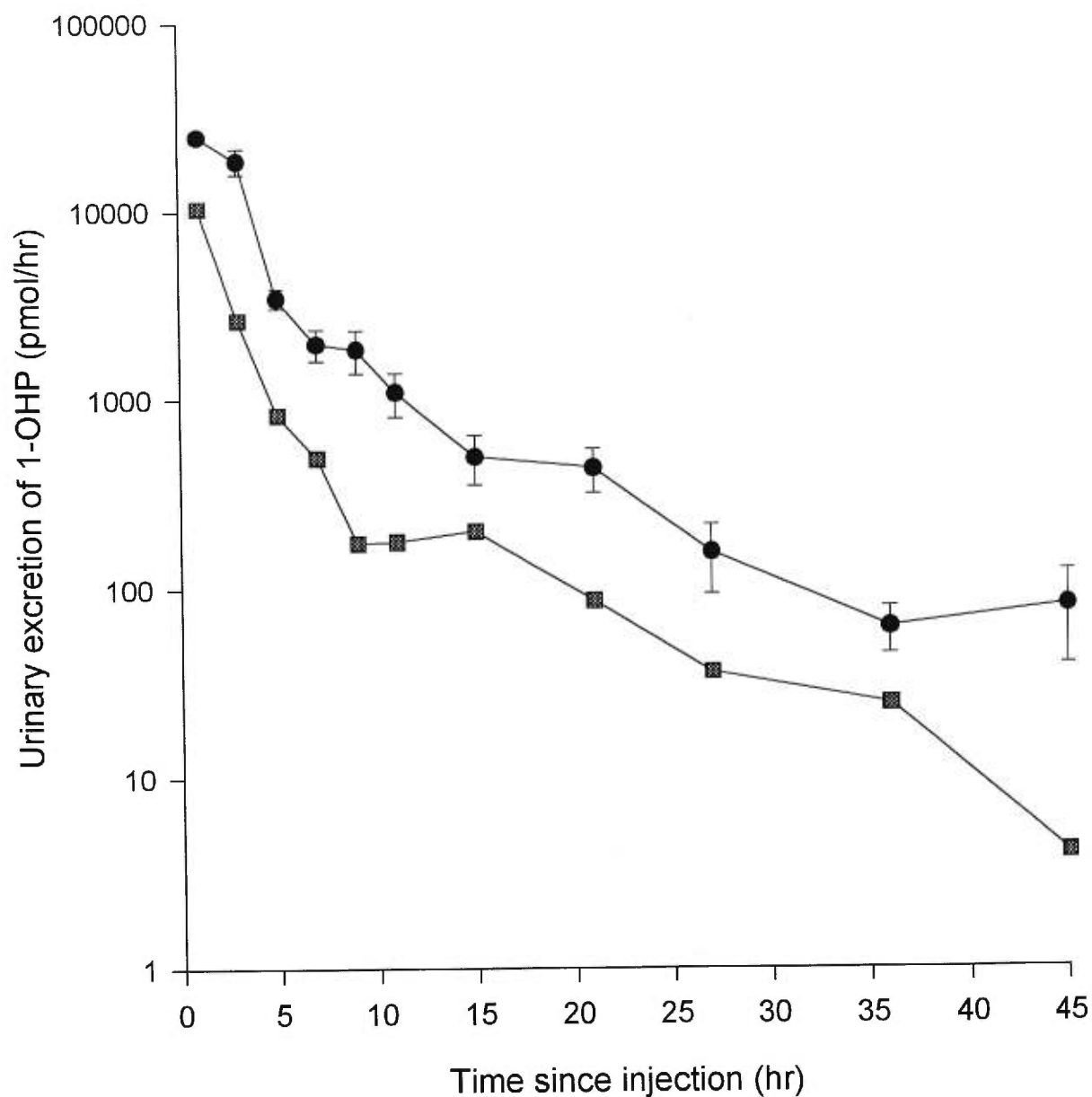


Figure 1. Urinary excretion kinetics of 1-OHP over a 48-hr collection period following an acute intravenous dose of 4 μmol 1-OHP/kg (●) or 15 μmol pyrene/kg (□) in male Sprague-Dawley rats. Each *point* represents mean excretion rate and corresponds to the midpoint of the sampling period and *vertical bars* are standard errors ($n=5$).

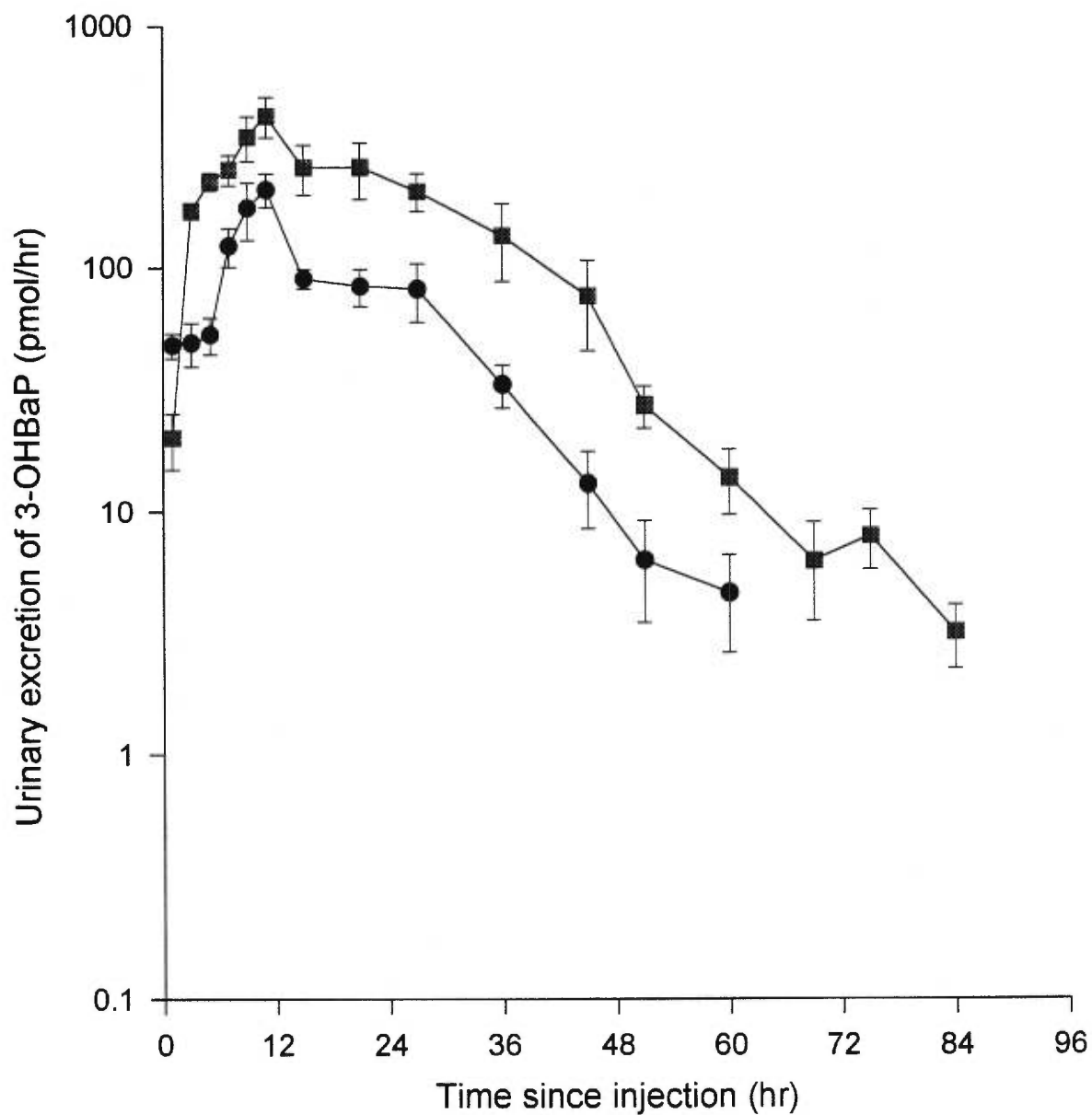


Figure 2. Urinary excretion kinetics of 3-OHBP over a 96-hr collection period following an intravenous dose of 2 $\mu\text{mol/kg}$ of a mixture of BaP metabolites (3-OHBP, 9-OHBP, 4,5-diolBaP, and 9,10-diolBaP) (●) or 40 $\mu\text{mol BaP/kg}$ (□) in male Sprague-Dawley rats. Each *point* represents mean and *vertical bars* are standard errors ($n= 5$).

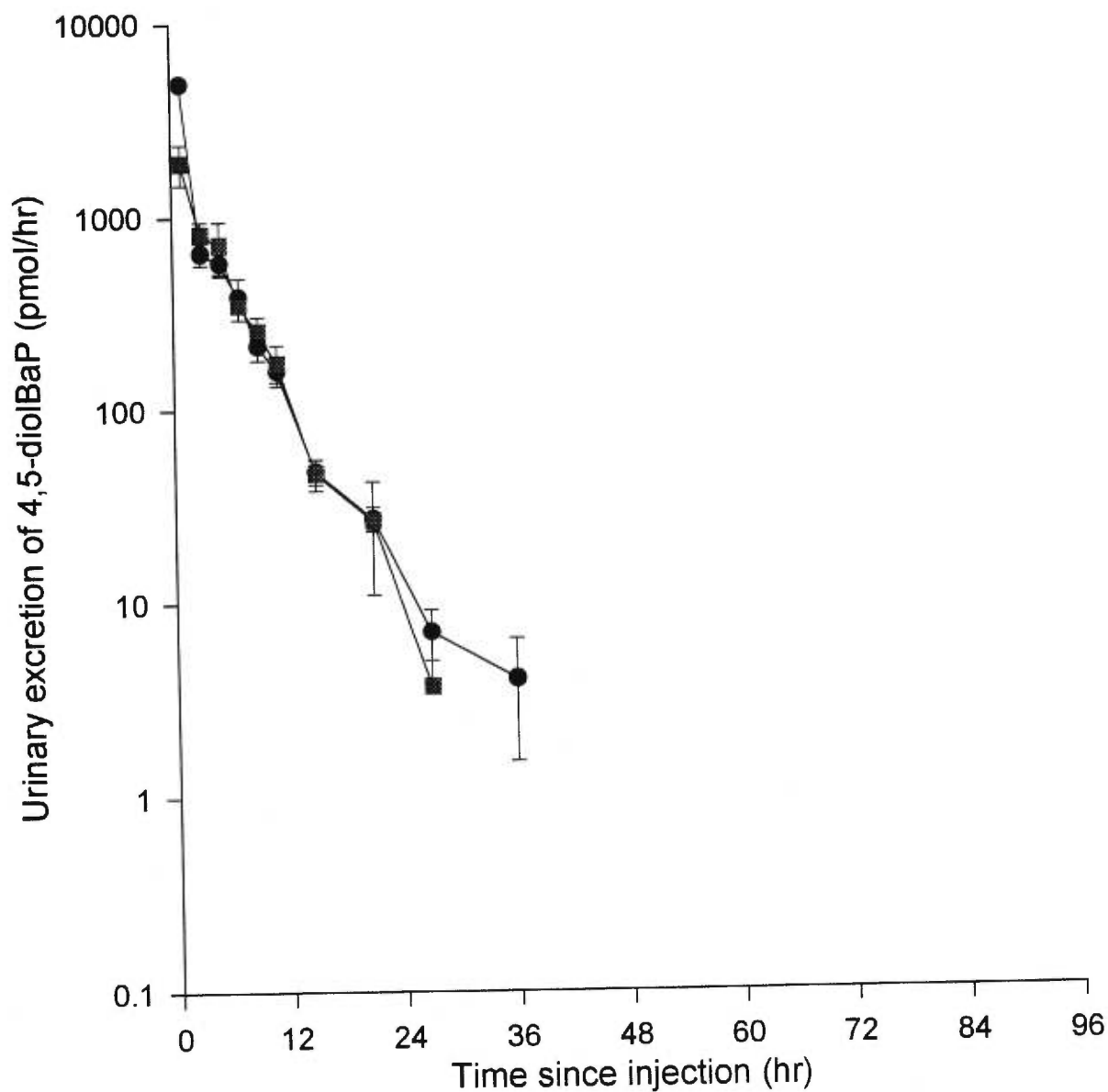


Figure 3. Urinary excretion kinetics of 4,5-diolBaP over a 96-hr collection period following an intravenous dose of 2 $\mu\text{mol/kg}$ of a mixture of BaP metabolites (3-OHBaP, 9-OHBaP, 4,5-diolBaP, and 9,10-diolBaP) (●) or 40 $\mu\text{mol BaP/kg}$ (□) in male Sprague-Dawley rats. Each *point* represents mean and *vertical bars* are standard errors ($n=5$).

TABLE 1

First order apparent half-lives of 1-OHP and BaP metabolites (3-OHBaP, 4,5-diolBaP, 9-OHBaP and 9,10-diolBaP) following intravenous administration of pyrene or BaP, respectively, or following injection of the metabolites.

Urinary metabolites	First order apparent half-life (hr)	
	Injection of metabolites ¹	Injection of the parent ² compound
1-OHP	6.6	6.9
3-OHBaP	7.6	8.1
4,5-diolBaP	3.6	3.1

¹ 1-OHP or an equimolar mixture of 3-OHBaP, 4,5-diolBaP, 9-OHBaP and 9,10-diolBaP

² Pyrene was injected for 1-OHP analysis; BaP was administered for BaP urinary metabolites analysis.

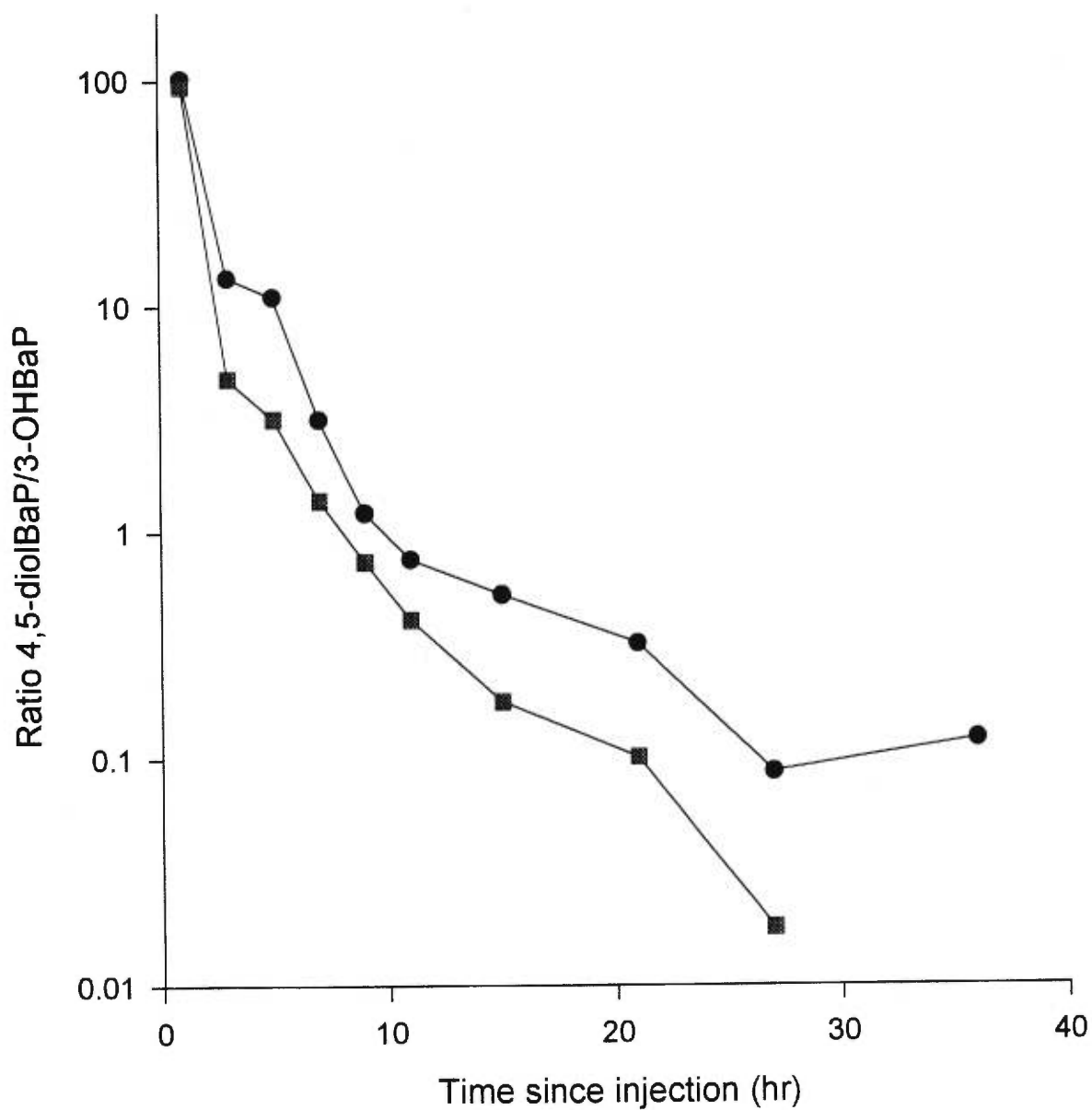


Figure 4. Variation in the ratio of 4,5-diolBaP/3-OHBaP urinary excretion with time since intravenous injection of 2 µmol/kg of a mixture of BaP metabolites (3-OHBaP, 9-OHBaP, 4,5-diolBaP, and 9,10-diolBaP) (●) or 40 µmol BaP/kg (□) in male Sprague-Dawley rats. Mean values are represented.

TABLE 2

Mean percentage of the dose recovered as 1-OHP over a 48-hr urine collection period or as BaP metabolites (3-OHBaP, 4,5-diolBaP, 9-OHBaP and 9,10-diolBaP) over a 96-hr sampling period following intravenous administration of pyrene or BaP, respectively, or following injection of the metabolites.

Metabolites	Mean % of the dose excreted as the various urinary metabolites over the entire urine collection period		Ratio B/A
	Injection of the metabolites ¹	Injection of the parent compound ²	
	A	B	
1-OHP	10.5	0.7	0.07
3-OHBaP	0.7	0.1	0.14
4,5-diolBaP	3.1	0.09	0.03
9-OHBaP	0.04	0.002	0.05
9,10-diolBaP	0.02	0.0001	0.005

¹ 1-OHP or an equimolar mixture of 3-OHBaP, 4,5-diolBaP, 9-OHBaP and 9,10-diolBaP

² Pyrene was injected for 1-OHP analysis; BaP was administered for BaP urinary metabolites analysis.

DISCUSSION

Measurements of urinary 1-OHP in spot urine samples of workers or non occupationally exposed individuals are used to estimate exposure to PAHs (Hansen *et al.*, 1994; Ferreira, Jr. *et al.*, 1994; van Rooij *et al.*, 1994). The current study was therefore initiated to further document the toxicokinetic profile of this bioindicator in order to better understand the significance of a measurement at a specific time point. Furthermore, since 1-OHP is used as a bioindicator of exposure to total PAHs (Jongeneelen *et al.*, 1990; Tolos *et al.*, 1990; Zhao *et al.*, 1990; Zhao *et al.*, 1992), it was thought interesting to compare the toxicokinetic behavior of urinary metabolites of another model PAH of greater health concern, BaP. Finally, previous studies suggested at least two elimination phases in the detailed excretion kinetics of 1-OHP following oral and dermal exposure to pyrene in humans (Viau *et al.*, 1995a; Viau and Vyskocil, 1995) or intravenous, oral and cutaneous administration of pyrene in rats (Dodd and Viau, unpublished). Results of an earlier study looking at the detailed excretion kinetics of urinary 3-OHBP following intraperitoneal administration of BaP also showed at least two steps of elimination (Bouchard and Viau, 1995). It was therefore decided to administer the metabolites of pyrene and BaP themselves to verify if phase I biotransformation was a rate limiting-step in the excretion kinetics of 1-OHP and BaP metabolites following administration of the parent compounds.

It was observed that the kinetic profiles after administration of the parent compound or the metabolites were comparable showing that phase I biotransformation was not the rate-limiting step in the excretion kinetics of 1-OHP following administration of pyrene, and in those of 3-OHBP and

4,5-diolBaP after BaP dosing. Considering similar excretion profiles of 1-OHP or of 3-OHBaP following injection of the parent compounds and the metabolites, it appears reasonable to speculate that metabolites themselves could distribute in some storage compartment. Moreover, Withey *et al.* (1992; 1993) reported that pyrene and BaP metabolites accumulate in various tissues following administration of radiolabelled pyrene or BaP by calculating the difference between a measurement of total radioactivity in tissues and the specific measurement of the parent compounds by HPLC. Interestingly, these authors also noted that, in general, for a given dose of the PAH, levels of BaP metabolites were significantly higher than those of pyrene metabolites. Additionally, possible explanations for the observed differences in the early portion of 1-OHP and 3-OHBaP urinary excretion curves would be that conjugation or excretion of the conjugated metabolites may be the rate-limiting step.

It is also noteworthy that the excretion kinetics of 4,5-diolBaP appeared linear but since its elimination is much more rapid than that of 3-OHBaP or 1-OHP, it could be that the first phase was not apparent with the number of data points obtained in the first hours following administration.

Furthermore, different excretion kinetics for 3-OHBaP and 4,5-diolBaP were determined. First, 4,5-diolBaP was excreted much more rapidly than 3-OHBaP and almost 100% of the total amount of 4,5-diolBaP excreted over the 96-hr collection period was eliminated within the first 24 hr post-dosing. Differences in the elimination rates between 3-OHBaP and 4,5-diolBaP suggest that the ratio of these metabolites can yield information on the exposure profile i.e. contemporary exposure would give a higher 4,5-diolBaP/3-OHBaP ratio than past exposure. Secondly, the profiles of excretion were very

different. Maximum excretion rate of 4,5-diolBaP was reached immediately following dosing and values decreased linearly with time from that time point. On the other hand, maximum excretion rates of 3-OHBaP occurred only 10 hr post-dosing and was followed by a linear decline in the excretion rates. First order apparent half-life of 3-OHBaP for the 24 to 66 hr phase post-dosing was about twice that of 4,5-diolBaP for the 2 to 30 hr phase following injection. It is further interesting to note that the amount of 4,5-diolBaP excreted was four times that of 3-OHBaP after injection of the mixture of BaP metabolites (3.1 and 0.7 % of the administered dose for 4,5-diolBaP and 3-OHBaP, respectively) and molar fraction of the parent BaP dose excreted as 3-OHBaP was comparable to that of 4,5-diolBaP (0.1 and 0.09 %, respectively).

In a previous experiment in rats exposed to BaP by various routes of exposure, it was found that 3-OHBaP was excreted in greater amounts than 4,5-diolBaP in a 72-hr urine sampling period following intravenous injection of BaP (on the average 0.06 and 0.03 % for 3-OHBaP and 4,5-diolBaP, respectively) and administration of BaP by gavage (on the average, 0.06 and 0.02 % for 3-OHBaP and 4,5-diolBaP, respectively) (Bouchard and Viau, submitted). These values are somewhat lower than those reported in the present study. Differences observed may result from a variation in the diet. Indeed, in the previous report, rats were allowed free access to food and tap water whereas in the present study, D-glucose and saccharin were added to the drinking water to induce polyuria and allow for frequent urine collection. Furthermore, in the present study, food was removed during a period of 18 hr (i.e. 6 hr prior to injection and during the first 12 hr following administration).

It was also observed that initial step of 1-OHP and 3-OHBaP urinary excretion differed substantially.

Maximum excretion rate of 1-OHP occurred immediately following injection while 3-OHBaP peak values were only reached after 10 hr post-dosing. Although different behaviors were observed between 1-OHP and 3-OHBaP for the first step of elimination (from 0 to 12 hr for 1-OHP and from 0 to 10-24 hr for 3-OHBaP), the second phase of elimination (from 12 to 48 hr for 1-OHP and from 24 to 66 hr for 3-OHBaP) was similar between these two metabolites. Indeed, elimination appeared linear in this second portion of the elimination curves and first order apparent half-lives of 6.9 and 8.1 hr could be calculated for urinary 1-OHP and 3-OHBaP, respectively after injection of the parent compounds. Similarly, half-lives of 6.6 and 7.6 hr were evaluated for urinary 1-OHP and 3-OHBaP, respectively following administration of the metabolites. The similarities in the first order apparent half-life of these two metabolites for the latter phase of elimination suggest that 1-OHP could be a good surrogate for 3-OHBaP, 1-OHP being easily detectable in human urine contrary to 3-OHBaP.

It is also noteworthy that the excretion kinetics of 3-OHBaP observed after BaP intraperitoneal dosing (Bouchard and Viau, 1995) were different from those obtained in the present study after intravenous administration of BaP or BaP metabolites. In the earlier study, following intraperitoneal administration, a plateau persisting over at least 48 hr was rapidly reached following treatment, excretion decreased linearly between 52 and 76 hr (mean time point between two sampling) post-dosing and a first order apparent half-life of 6.7 hr could be calculated for this phase. A second phase was observed between 72 and 164 hr showing a first order apparent half-life of 25 hr. On the other hand, in the current study, after intravenous dosing, although maximum excretion was not reached immediately following treatment, a plateau persisting that long a period of time was not observed. However, the half-life of 6.7 hr observed for the first elimination phase after intraperitoneal dosing

was close to the value of 8.1 hr obtained after BaP intravenous injection. The second phase observed after intraperitoneal dosing and for which a half-life of 25 hr was determined, was not apparent after intravenous dosing. These findings suggest that intraperitoneal dosing favors the storage of BaP in abdominal fat, this BaP being slowly released with time as suggested by Withey *et al.* (1993). This aspect needs, however, to be further investigated. It would also be interesting to look at the excretion kinetics of 3-OHBaP following intraperitoneal injection of the metabolite itself to see if the plateau observed in the first step of elimination could be due to BaP enzymatic induction of its own metabolism.

As for excretion of 9-OHBaP and 9,10-diolBaP metabolites determined in the present study, only small levels were detected in urine after injection of BaP as well as after injection of the metabolites themselves suggesting that they are preferentially excreted in feces or undergo further metabolism before being excreted.

Furthermore, as expected, molar fraction of the dose excreted as the various metabolites following administration of the metabolites themselves was higher than after administration of the parent compounds. Nonetheless, only a small percentage of the metabolites injected were recovered in urine, these probably being excreted in feces mostly, as observed after administration of the parent compound (van de Wiel *et al.*, 1993; Jacob *et al.*, 1989). It is also noteworthy that the mean percentage of the dose excreted in urine as 1-OHP was higher than that excreted as 3-OHBaP when injecting either the parent compound or the metabolite which could be related to the greater biliary excretion of the higher molecular weight 3-OHBaP compared to 1-OHP. On the other hand, when

looking at BaP metabolites in urine, it is also interesting to note that the molar fraction of the dose excreted as the different BaP metabolites differed when injecting BaP as compared to the metabolites.

It should also be noted that molar fraction of the pyrene dose excreted as 1-OHP in rat urine (Table 2, column B) was much lower than that calculated in human volunteers exposed to pyrene (3.7%) (Viau *et al.*, 1995a; Viau and Vyskocil, 1995). This could be related to the fact that there is a threshold molecular weight for biliary excretion of organic anions below which little biliary elimination occurs. The glucurono-conjugate of 1-OHP has a molecular weight of 378, a value slightly above that which is usually considered as a threshold for biliary excretion in rats (325) (Smith, 1973), but lower than the threshold in humans (ca 500) (Klaassen and Watkins, 1994), which should result in a lower biliary excretion of the 1-OHP-glucuronide in humans. Other possible explanation for the differences in the quantitative urinary excretion of 1-OHP between rats and humans would be differences in the extent of phase I biotransformation of pyrene to 1-OHP. The interspecies differences observed do not appear to be related to a saturation of the excretion capacity of the rats since, in a previous study, a strong relationship between the dose and the urinary excretion of 1-OHP was found ($R^2 = 0.99$, $p < 0.001$) in rats exposed to intravenous doses of pyrene ranging from 0.5 to 50 $\mu\text{mol/kg}$ (Dodd and Viau, unpublished).

Finally, when injecting the metabolites, it can be considered that the molar fraction of the dose recovered in urine as 1-OHP or BaP metabolites represents the total amount of what can be excreted of these metabolites in urine, the rest being found in feces. Measuring the ratio: fraction of the pyrene dose excreted in urine as 1-OHP/ fraction of the 1-OHP dose excreted in urine as 1-OHP or the ratio:

fraction of the BaP dose excreted in urine as BaP metabolites/ fraction of BaP metabolites dose excreted in urine as BaP metabolites would inform on the amount of pyrene and BaP that is biotransformed and excreted as 1-OHP or BaP metabolites. Results show that 1-OHP is formed in smaller amounts than 3-OHBaP (Table 2). 3-OHBaP is formed in greater amounts than 4,5-diolBaP and 9-OHBaP. The small ratio values obtained may be explained by the fact that the largest part of the dose remains accumulated in the organism. Laparé *et al.* (1994) suggested a binding of pyrene to specific receptors in the liver and lung (i.e. four fifth of the dose is found bound to proteins) as observed in the case of other aromatics (Andersen *et al.*, 1993).

In conclusion, differences in the detailed urinary excretion kinetics of 1-OHP and BaP metabolites were observed. Furthermore, results show that urinary excretion kinetics of 1-OHP or of BaP metabolites were similar following administration of the parent compound and the metabolites. These findings demonstrate that phase I biotransformation is not the rate-limiting step in the excretion kinetics of these urinary bioindicators following administration of the parent compounds. It can also be concluded that a measure of the concentration ratio of 4,5-diolBaP/3-OHBaP could inform on the exposure profile. Finally, similarities in the first order apparent half-life of 1-OHP and 3-OHBaP for the latter phase of elimination suggest that 1-OHP could be a good surrogate for 3-OHBaP.

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ARTICLE 3

Urinary and biliary excretion kinetics of 1-hydroxypyrene following intravenous and oral administration of pyrene in rats

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Abstract

The urinary and biliary excretion kinetics of 1-hydroxypyrene (1-OHP) were compared in male Sprague-Dawley rats exposed intravenously and orally to 1.5, 5, 15, 50 and 100 $\mu\text{mol/kg}$ of pyrene. Urine and bile samples were collected at 6-h intervals up to 24 h. Results showed kinetics of 1-OHP were similar for both biliary and urinary excretion whatever the administered dose or exposure route. Furthermore, the time-course of 1-OHP excretion in either bile or urine following intravenous dosing resembled that observed after oral administration. In addition, the exposure route and dose had no significant effect on the fraction of dose recovered in urine and bile as 1-OHP after 6, 12, 18, and 24 h post-dosing. Following intravenous injection of 1.5, 5, 15, 50 and 100 $\mu\text{mol/kg}$ of pyrene, mean cumulative percent of dose excreted as 1-OHP in urine over 24 h ranged from 1.7 to 3.2% while biliary values ranged from 6.5 to 9.5%. Correspondingly, after oral administration, on the average, 2.6 to 3.3% of dose was excreted as 1-OHP in urine and 7.9 to 10.9% was recovered in bile. Overall, the linear dose-excretion relationship following either exposure routes supports the usefulness of 1-OHP in urine as a bioindicator of PAH exposure. Results further suggest that tissue uptake and distribution of intravenously and orally administered pyrene proceeds similarly. By comparing these data with predicted values from a PBPK model for pyrene, it also appears that some part of pyrene dose remains in the organism after 24 h and that metabolites other than 1-OHP as measured in the current study are present in significant proportion in urine and feces.

Keywords: 1-Hydroxypyrene; Urinary excretion kinetics; Biliary excretion kinetics; Polycyclic aromatic hydrocarbons; Pyrene

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a broad class of ubiquitous contaminants originating from the incomplete combustion or pyrolysis of organic matter (Nikolaou et al., 1984) and present as complex mixtures (Jongeneelen et al., 1988; Petry et al., 1994). Biological monitoring, which measures the absorbed dose, represents a useful tool for risk assessment resulting from PAH exposure provided there is an adequate knowledge of the toxicokinetics of PAHs and their metabolites in the organism (Lauwerys and Hoet, 1993; ACGIH, 1991).

A few years ago, Jongeneelen et al. (1986, 1987) proposed 1-hydroxypyrene (1-OHP) in urine as a bioindicator of the overall exposure to PAHs. 1-OHP is a major metabolite of pyrene (Boylard and Sims, 1964; Jacob et al., 1982; Keimig et al., 1983) which is found in important proportion (1-10%) in most PAH mixtures (Buchet et al., 1992; Roussel et al., 1992). Given the availability of a straightforward and sensitive method for 1-OHP determination (Jongeneelen et al., 1985) which allows to easily quantify this metabolite in human urine, including control populations (Viau et al., 1995), many researchers have resorted to this measurement for the biological monitoring of exposure to PAHs in the workplace and in the general environment (Levin, 1995; Gilbert and Viau, 1997).

Adequacy of the use of 1-OHP as a bioindicator of total PAHs is dependent, on the one hand, on a good relationship between exposure concentrations of pyrene and those of total PAHs and, on the other hand, on the linear relationship between pyrene dose and 1-OHP urinary excretion. Saturation of absorption, metabolism (phase I and II biotransformation enzymes) and excretion (urinary and

biliary transport systems) can result in non-linear dose-excretion curves. Since it appears that a large part of ^{14}C -pyrene dose is excreted via the bile and hence the feces in rats (Withey et al., 1991, 1992, 1993a) and since biliary transport of anions is an active transport system, it is thus subject to a possible saturation (Sathirakul et al., 1994) which could result in an overflow of 1-OHP towards the plasma and consequently an increased urinary excretion. It therefore appears pertinent to evaluate the relative importance of biliary and urinary excretion of 1-OHP in particular with relation to the exposure dose and the administration route.

Such a saturation of biliary transport of the sum of benzo(a)pyrene (BaP) metabolites in rats has been demonstrated from ^{14}C -BaP doses of $3.2 \mu\text{mol/kg}$ and above (Kotin et al., 1959). Withey et al. (1991) have also reported that following intravenous and oral administration of 2 mg/kg of ^{14}C -pyrene, respectively 37% and 12% of dose was recovered in the bile over 6 h post-dosing. Increasing the dose to 9 mg/kg caused a decrease of these respective percentages to 27% and 8%. This question needs to be addressed, especially since the glucurono-conjugate of 1-OHP, which is the main excretory form of 1-OHP in feces of rats (Boyland and Sims, 1964), has a molecular weight of 378, a value slightly above that which is usually considered as a threshold for biliary excretion (i.e. 325) in the rat (Klaassen and Rozman, 1991).

The objective of the present study was therefore to compare the urinary and biliary excretion kinetics of 1-OHP following intravenous and oral administration of five pyrene doses.

2. Materials and methods

2.1. Chemicals

Pyrene (99% purity) was purchased from Sigma-Aldrich (Oakville, ON, Canada) and used as received. Reference standard of 1-OHP (>99% purity) was obtained from the NCI Chemical Carcinogen Reference Standards distributed by Midwest Research Institute (Kansas, MO) and used as received. Ketamine and xylazine were purchased from CDMV inc. (St-Hyacinthe, Qc, Canada). β -glucuronidase/arylsulfatase (100 000 Fishman U/ml and 800 000 Roy U/ml from *Helix pomatia*) was purchased from Boehringer Mannheim GmbH (Laval, Qc, Canada). Alkamuls EL-620 (formerly emulphor 620) was kindly provided by Rhone-Poulenc (St-Hyacinthe, Qc, Canada). HPLC grade methanol was purchased from VWR-Canlab (Mississauga, ON, Canada). Ascorbic acid was obtained from Baker Analyzed Biochemical (J.T. Baker Chem. Co, Phillipsburg, NJ).

2.2. Animals

Male Sprague-Dawley rats (Charles River Canada Inc., St-Constant, Quebec) of 200 to 250 g were used. The animals were kept in individual metabolic cages. Lighting was maintained on a 12-h light-dark cycle and room temperature was kept at $22 \pm 3^\circ\text{C}$. Animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

2.3. *Animal treatment*

Rats for which bile was collected were anesthetized with ketamine (80 mg/kg) and xylazine (10 mg/kg). This anaesthesia has been shown to be without consequences on urine output and bile volumes (Fleck and Barth, 1990). The bile duct was cannulated using a polyethylene tubing Model PE-10 (VWR-Canlab, Mississauga, ON, Canada). The cannula was inserted under the skin toward the back of the animal to exit in the neck area. It was then inserted into a steel spring of 300 mm connected to a 20 mm PE 320 tubing which was then tied to the skin. The other extremity of the spring was anchored to the metabolic cage. This permitted the animal to move freely in the cage while preventing damage to the tubing (D'Amour and Charbonneau, 1992). The animals were allowed to recover during the 24-h period following surgery. Only rats with normal bile flow were used in the experiment.

During their acclimatization period, rats received drinking water containing glucose (40 g/l) and saccharin (1.5 g/l). This induced a polydipsic behavior and thus an aqueous diuresis allowing for frequent urine collections. Rats were also provided with food during that period of time. Following cannulation of the biliary duct, rats had access to food and water containing glucose (40 g/l), saccharin (1.5 g/l) and electrolytes (NaCl 0.9% and KCl 0.05%). Food was however removed 6 h prior to administration of pyrene.

These rats were then exposed intravenously (by a tail vein) or orally (by gastric intubation) to a 1.5, 5, 15, 50 or 100 $\mu\text{mol/kg}$ dose of pyrene ($n=4$ per dose for each administration route). Pyrene was

dissolved in 20% emulphor: 80% isotonic glucose solutions. For intravenous injection, one ml of solution was injected per kilogram of body weight when administering the 1.5, 5 and 15 $\mu\text{mol/kg}$ dose and 3 ml of solution was injected per kilogram of body weight when administering the 50 and 100 $\mu\text{mol/kg}$ dose of pyrene to ensure complete solubility of pyrene. For the oral administration, 5 ml of solution was injected per kilogram of body weight for all doses. Rats were treated at approximately 03:00 pm to take advantage of the active part of their circadian cycle.

In a complementary experiment aiming at determining the effect of cannulation on the urinary excretion of 1-OHP, groups of four rats without and with biliary cannulas were exposed intravenously to 1.5 $\mu\text{mol/kg}$ of pyrene by a tail vein. One ml of solution was injected per kilogram of body weight. These rats were subjected to the same protocol of administration of food and water supplemented with sugar and electrolytes as previously mentioned.

A second complementary experiment was conducted to establish the effect of providing rats with drinking water containing both sugar (glucose and saccharin) and electrolytes (NaCl and KCl) or sugar only on the urinary excretion of 1-OHP. Non cannulated rats were used and separated in three different groups of four rats. First group received normal water (tap water) and food during their acclimatization period, and food was removed 6 h prior to dosing. In the second group, tap water was replaced by sweetened water. In the third group, the protocol described above for cannulated rats was used. In each case, rats were exposed intravenously to 5 $\mu\text{mol/kg}$ of pyrene by a tail vein. One ml of solution was injected per kilogram of body weight.

In a third complementary experiment, the effect of duration of food-fasting on the urinary excretion of 1-OHP in rats drinking either normal water, sweetened water or sweetened water with added electrolytes was investigated. Non cannulated rats were also used and separated in three groups: rats receiving normal water, sweetened water or sweetened water with electrolytes. Each group was divided in subgroups of four rats for which food was removed 6, 12, 24 or 48 (except the group drinking normal water) h before dosing. Rats were exposed intravenously to 5 $\mu\text{mol/kg}$ of pyrene by a tail vein. One ml of solution was injected per kilogram of body weight.

2.4. Urine and bile sampling

In every experiment, urine and bile samples were collected at 6-h intervals over a 24-h period following administration (i.e. 0-6, 6-12, 12-18, 18-24 h post-dosing) except in the experiment aiming to determine the effect of duration of food-fasting on the urinary excretion of 1-OHP in rats provided either with normal drinking water, sweetened water or sweetened water with added electrolytes. In this last case, 24-h urine samples were collected following treatment. Control samples were also collected during a 4-h period preceding administration.

2.5. Urine analysis

Urine samples were collected over thymol and frozen at -20°C until analysis. Samples were treated using the method described by Jongeneelen et al. (1987) which involves the determination of the sum of unconjugated and conjugated 1-OHP after enzymatic hydrolysis of the glucurono- and sulfo-

conjugated metabolites.

2.6. Bile analysis

Samples were frozen at -20°C until analysis. Samples were analyzed according to a method adapted from that described by Chipman et al. (1981) for the analysis of BaP metabolites in bile. Briefly, bile samples (200 μl) were buffered with 4 ml of 0.1 M sodium acetate solution, pH 5.0 and incubated overnight with 1 μl of β -glucuronidase/arylsulfatase in a shaking bath at 37°C . Metabolites were then extracted with 2×4 ml of ethyl acetate saturated with water and centrifuged at 1000 rpm for 20 min. Solvent was evaporated to dryness under nitrogen flow at 50°C and samples were redissolved in 2 ml of methanol containing naphthalene (1 mg/l). Efficiency of the extraction of hydrolyzed metabolites was determined using untreated rat bile samples spiked with 12.5, 25, 50 and 100 pmol of authentic 1-OHP reference standard. Recovery from 12 spiked samples was $74 \pm 8\%$ (mean \pm S.D.). Results presented in this article have been corrected for recovery.

It should be noted that a preliminary experiment was conducted to establish the optimal conditions for the hydrolysis of the glucurono- and sulfo-conjugates of 1-OHP in bile. It has also been determined, by comparing biliary concentrations of 1-OHP obtained in samples (aliquots of pooled bile from rats treated with pyrene) subjected or not to enzymatic hydrolysis ($n=3$ per group), that free 1-OHP in bile represented on the average 16% of total 1-OHP (thus of the sum of free and sulfo and glucurono-conjugated 1-OHP).

2.7. HPLC analysis of urine and bile

Samples analysis was performed using a high performance liquid chromatographic (HPLC) system composed of an automatic injector AS-100 (Bio-Rad, Richmond, CA), a binary pump model 250 (Perkin-Elmer, Buckingham, England), a LC-18 Supelcosil 250×4.6 mm column (Supelco, Oakville, Ontario), a fluorescence detector LS-40 (Perkin-Elmer), a PE Nelson 900 interface (Perkin-Elmer) and a PE Nelson turbochrom 3 integration system. Separation was performed in 25 min using a 71%:29% methanol:water mobile phase. Recovery of the analyte and reproducibility of the analysis were improved by the addition of ascorbic acid (1 mg/l) to the methanol eluent (Bouchard et al., 1994). Injection volume was 20 μ l. Column temperature was 32°C and flow rate of 0.8 ml/min. Excitation and emission wavelengths were 242 and 388 nm, respectively.

2.8. Statistics

Values are reported as mean \pm S.D. One-way analysis of variance (ANOVA) was performed to test the effect of the dose and the administration route on the fraction of dose recovered as 1-OHP in either urine or bile for each interval of time (0-6, 0-12, 0-18 and 0-24 h). The level of significance was set at $P = 0.05$. In the case of a difference among the group means, Bonferroni post-test was conducted to determine if pairs of values were significantly different from each other. Linear regressions were also performed to determine the relationship between dose and total excretion of 1-OHP in urine or bile following either intravenous or oral pyrene administration and R^2 and P values were calculated. Unpaired-t test was conducted to test the differences in the mean excretion values

of 1-OHP between rats without or with biliary cannulas for the intervals of time 0-6, 0-12, 0-18 or 0-24 h post-dosing. One-way ANOVA and unpaired-t test were performed to test the differences in 1-OHP urinary excretion in rats drinking tap water only, water with added sugar or water with added sugar and electrolytes for which food was removed 6 h prior to dosing. One-way ANOVA and unpaired-t test were also used to test the effect of duration of food-fasting on the urinary excretion of 1-OHP in rats provided with either normal drinking water, sweetened drinking water or sweetened water supplemented with electrolytes. The level of significance was set at 0.05.

3. Results

3.1. Urinary and biliary excretion of 1-OHP following intravenous and oral administration of pyrene

Figs. 1 and 2 compare the time course of 1-OHP urinary (A) and biliary (B) excretion following intravenous and oral administration of pyrene, respectively, at five different doses. Kinetics of 1-OHP in bile, following either intravenous or oral dosing, compared well with those obtained in urine independently of the administered dose. On the other hand, time-course of 1-OHP excretion in either bile or urine following intravenous injection were similar to those observed after oral administration. With the number of time points available, only a crude estimation of the elimination half-lives could however be made. For comparison purpose, Fig. 1 also includes the detailed urinary excretion profile of 1-OHP described in a previous report for non cannulated rats exposed intravenously to 5 $\mu\text{mol/kg}$ of pyrene where urine samples were collected at frequent intervals over a 48-h period following administration. It can be seen that urinary excretion kinetics obtained in the current report were consistent with that previously described. In the latter case, a biphasic elimination was depicted with a more rapid phase followed by a slower phase (12 to 42 h post-dosing) for which a first order apparent elimination half-life of 6.5 ± 1.1 h (mean \pm S.D.) was calculated.

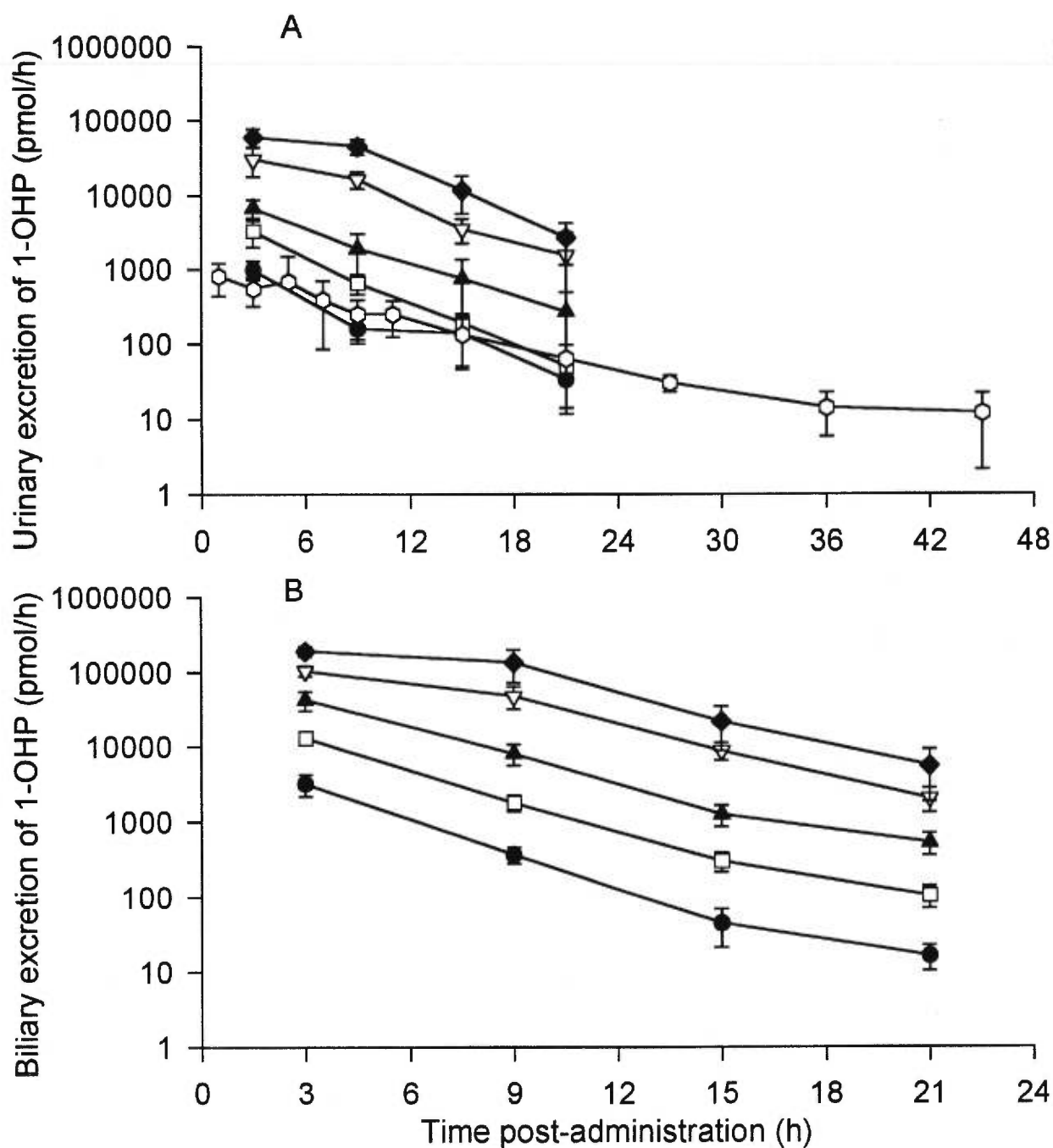


Fig. 1. Urinary (A) and biliary (B) excretion patterns of 1-OHP following intravenous administration of 1.5 (●), 5 (□), 15 (▲), 50 (▽) and 100 (◆) $\mu\text{mol/kg}$ of pyrene in rats with biliary cannulas (mean \pm S.D. for 4 rats). Urinary excretion profile of 1-OHP following intravenous administration of 5 $\mu\text{mol/kg}$ of pyrene in non cannulated rats (◇) (mean \pm S.D. for 6 rats).

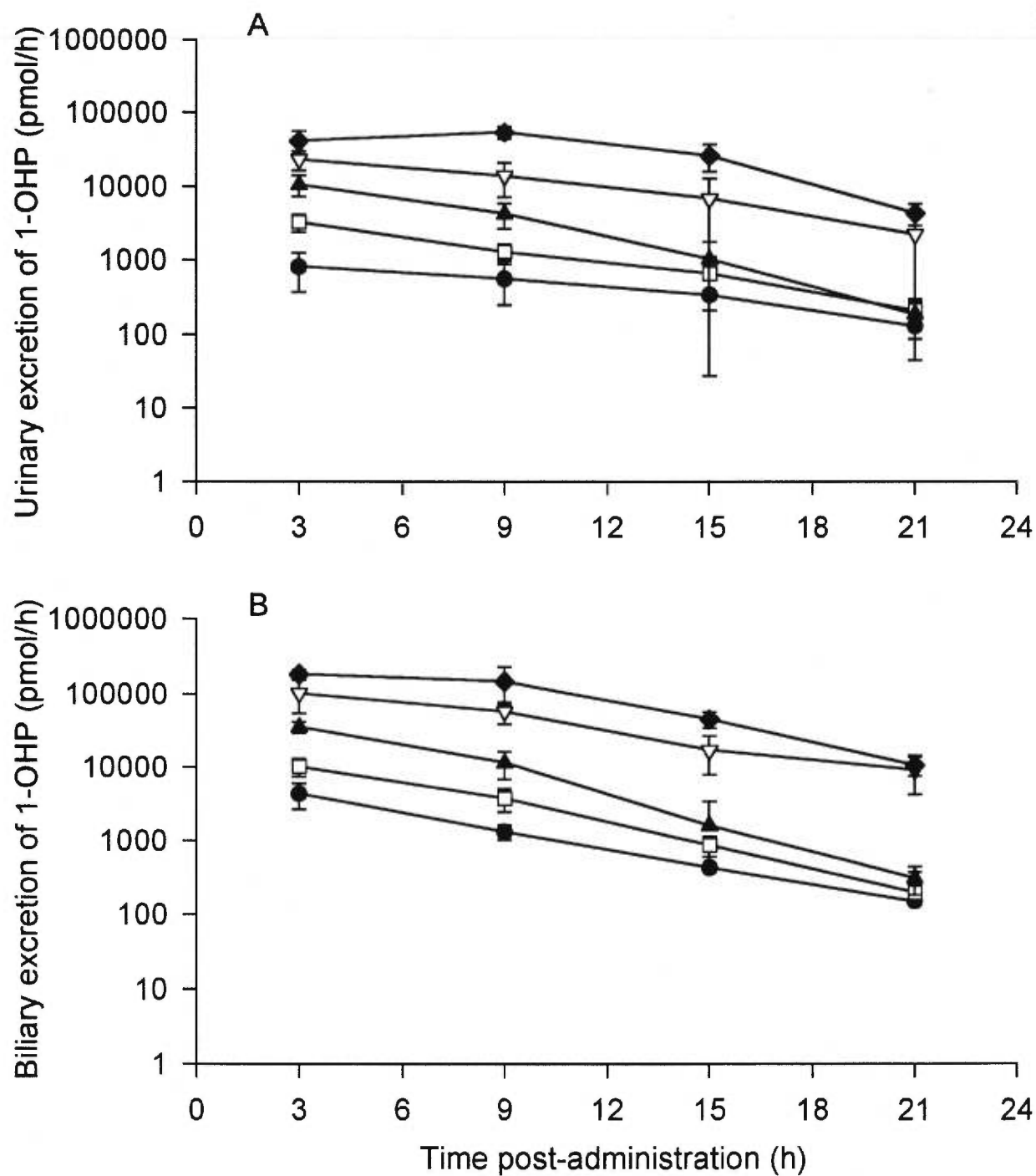


Fig. 2. Urinary (A) and biliary (B) excretion patterns of 1-OHP following oral administration of 1.5 (●), 5 (□), 15 (▲), 50 (▽) and 100 (◆) $\mu\text{mol/kg}$ of pyrene in rats with biliary cannulas (mean \pm S.D. for 4 rats).

3.2. Cumulative 1-OHP urinary and biliary excretion following intravenous and oral administration of pyrene

Table 1 describes the cumulative percentage of the pyrene dose excreted as 1-OHP in urine and bile after 6, 12, 18 and 24 h following intravenous and oral administration. A lack of significant effect of dose and of the administration route on the time course of 1-OHP cumulative excretion in both urine and bile was observed. It was also readily seen that amounts of 1-OHP excreted in bile were more important than those excreted in urine. On the average, following intravenous administration of 1.5, 5, 15, 50 and 100 $\mu\text{mol/kg}$ of pyrene, biliary excretion values were approximately 3 to 5 times those obtained in urine. Following oral administration, biliary values were about 3 to 4 times those in urine. However, the sum of 1-OHP urinary and biliary cumulative excretion over 24 h represented only a small fraction of the pyrene dose (less than 15%).

3.3. Relationship between the administered dose and 1-OHP excretion in urine and bile following intravenous and oral administration of pyrene

Fig. 3 depicts the linear dose-excretion relationship observed in urine as well as in bile over 24 h following intravenous and oral administration of pyrene. Following intravenous injection, correlation coefficients (R^2) of 0.993 with P value = 0.0002 and R^2 of 0.998 with $P < 0.0001$ were obtained for the urinary and biliary route, respectively. Accordingly, following oral administration, R^2 of 0.981 with $P = 0.0011$ and R^2 of 0.999 with $P < 0.0001$ were calculated for urine and bile.

Table 1

Cumulative urinary and biliary excretion of 1-OHP following intravenous and oral administration of 1.5, 5, 15, 50 and 100 $\mu\text{mol/kg}$ of pyrene in cannulated rats

Exposure route	Biological fluid	Dose ($\mu\text{mol/kg}$)	Cumulative 1-OHP excretion (percentage of dose) mean \pm S.D. for 4 rats ^a			
			Time interval post-dosing (h)			
			0-6	0-12	0-18	0-24
Intravenous	Urine	1.5	1.8 \pm 0.5	2.1 \pm 0.6	2.3 \pm 0.5	2.4 \pm 0.5
		5	1.7 \pm 0.7	2.1 \pm 0.7	2.2 \pm 0.8	2.2 \pm 0.8
		15	1.2 \pm 0.3	1.6 \pm 0.4	1.7 \pm 0.5	1.7 \pm 0.5
		50	1.6 \pm 0.6	2.4 \pm 0.5	2.6 \pm 0.4	2.6 \pm 0.4
		100	1.6 \pm 0.4	2.8 \pm 0.3	3.1 \pm 0.3	3.2 \pm 0.3
	Bile	1.5	5.7 \pm 1.9	6.4 \pm 1.9	6.5 \pm 1.9	6.5 \pm 1.9
		5	6.8 \pm 0.6	7.8 \pm 0.4	7.9 \pm 0.4	8.0 \pm 0.4
		15	7.6 \pm 2.3	9.0 \pm 2.5	9.2 \pm 2.6	9.3 \pm 2.6
		50	5.4 \pm 0.9	7.8 \pm 1.3	8.3 \pm 1.4	8.4 \pm 1.4
		100	5.2 \pm 0.9	8.8 \pm 2.2	9.4 \pm 2.5	9.5 \pm 2.5
Oral	Urine	1.5	1.4 \pm 0.8	2.4 \pm 1.1	3.1 \pm 1.5	3.3 \pm 1.6
		5	1.7 \pm 0.5	2.4 \pm 0.6	2.8 \pm 0.7	2.9 \pm 0.7
		15	1.9 \pm 0.6	2.6 \pm 0.8	2.8 \pm 0.8	2.8 \pm 0.8
		50	1.3 \pm 0.4	2.0 \pm 0.6	2.4 \pm 0.9	2.6 \pm 1.1
		100	1.1 \pm 0.4	2.5 \pm 0.5	3.1 \pm 0.4	3.2 \pm 0.4
	Bile	1.5	7.6 \pm 2.8	9.9 \pm 2.7	10.7 \pm 3.5	10.9 \pm 3.8
		5	5.4 \pm 1.3	7.4 \pm 1.9	7.8 \pm 1.9	7.9 \pm 1.9
		15	6.2 \pm 1.2	8.2 \pm 1.8	8.5 \pm 2.0	8.5 \pm 2.0
		50	5.6 \pm 2.5	8.8 \pm 2.3	9.7 \pm 2.3	10.2 \pm 2.3
		100	4.8 \pm 0.6	8.6 \pm 2.7	9.8 \pm 2.5	10.1 \pm 2.5

^aANOVA showed that exposure dose and route had no statistically significant effect on 1-OHP mean excretion values in urine or bile for either interval of time post-dosing; $P > 0.05$.

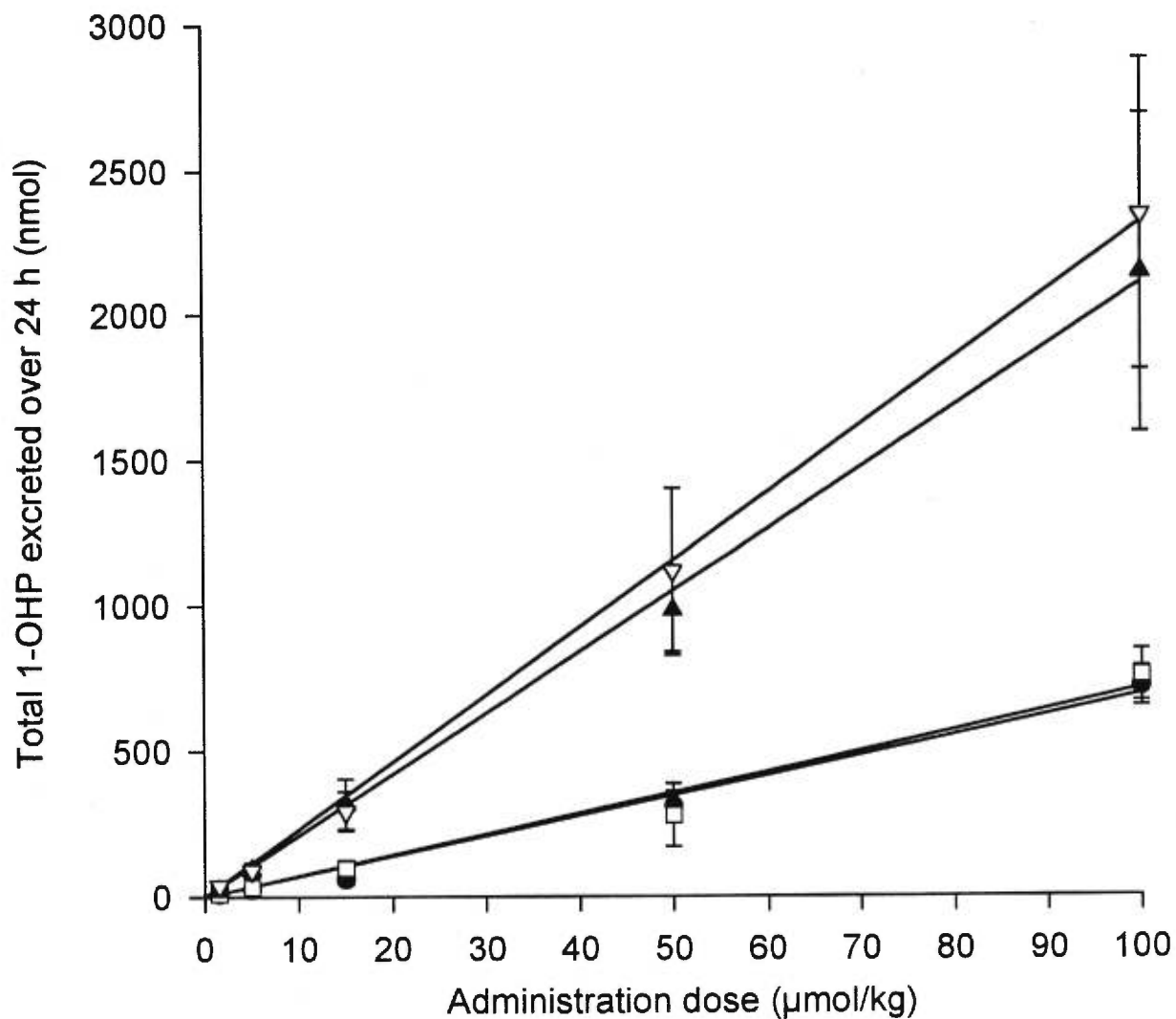


Fig. 3. Dose-dependent excretion of 1-OHP in urine and bile over 24 h following intravenous and oral administration of 1.5, 5, 15, 50 and 100 µmol/kg of pyrene in rats with biliary cannulas (mean ± S.D. for 4 rats). ●= 1-OHP in urine following intravenous administration; □= 1-OHP in urine following oral administration; ▲= 1-OHP in bile following intravenous administration; ▽= 1-OHP in bile following oral administration.

3.4. Effect of cannulation on the urinary excretion of 1-OHP following intravenous administration of pyrene

Table 2 shows that cannulation of the biliary duct did not result in a significant effect on the cumulative percentage of the pyrene dose excreted in urine as 1-OHP after 6, 12, 18 and 24 h post-dosing. Molar fraction of the pyrene dose excreted as 1-OHP in urine over 24 h was $2.4 \pm 0.5\%$ in rats with biliary cannulas exposed to $1.5 \mu\text{mol/kg}$ of pyrene as compared to $3.2 \pm 1.0\%$ in rats without cannulas exposed to the same dose.

3.5. Effect of addition of sugar or sugar and electrolytes on the urinary excretion of 1-OHP in rats without biliary cannulas

Table 3 shows the effect of adding glucose and saccharin to the drinking water or glucose, saccharin and electrolytes on 1-OHP urinary excretion after 6, 12, 18, and 24 h in rats without biliary cannulas for which food was removed 6 h prior to dosing. It was observed that amounts of 1-OHP excreted in the urine of rats drinking sweetened water with added electrolytes were not significantly different from those recovered in urine of rats drinking sweetened water only. In contrast, rats drinking ordinary water had significantly lower excretion values after 6, 12, 18 and 24 h than the latter two groups. Indeed, percentage of the pyrene dose excreted as 1-OHP in urine over 24 h was $3.0 \pm 1.5\%$ in rats drinking water with added sugar and electrolytes and $2.9 \pm 0.6\%$ in rats drinking sweetened water only as compared to $0.7 \pm 0.3\%$ in rats drinking ordinary water. Nonetheless, 1-OHP urinary excretion kinetics appeared similar in all three groups. When calculating the ratio of mean percentage

of dose excreted in urine as 1-OHP in rats supplied with sweetened water or sweetened water with added electrolytes to mean percentage obtained in rats drinking tap water only, a constant value was obtained for the different time points (that is 6,12,18 and 24 h post-dosing).

3.6. Effect of supplying sweetened drinking water or sweetened water with added electrolytes and of the duration of food-fasting prior to dosing on the urinary excretion of 1-OHP in rats without biliary cannulas

Fig. 4 shows that whatever the duration of food-fasting, a significant increase in total 1-OHP urinary excretion over 24 h post-dosing was depicted in rats given sweetened water with added electrolytes as compared to rats drinking plain tap water. Significant differences in 1-OHP total urinary excretion between rats provided with sweetened water as compared to sweetened water with added electrolytes were observed when removing food 12 and 24 h prior to dosing whereas similar values were obtained for those two groups when food was removed 6 and 48 h before injection.

It can furthermore be observed that when supplying ordinary drinking water or sweetened drinking water with added electrolytes, duration of food-fasting prior to dosing did not in general influence the urinary excretion of 1-OHP in non cannulated rats. However, when providing sweetened water, duration of food-fasting tended to have an effect on 1-OHP excretion although the differences were not statistically significant.

Table 2

Urinary excretion of 1-OHP following intravenous administration of 1.5 $\mu\text{mol/kg}$ of pyrene in rats without and with biliary cannulas

Rats	Cumulative 1-OHP excretion (percentage of dose) mean \pm S.D. for 4 rats ^a			
	Time interval post-dosing (h)			
	0-6	0-12	0-18	0-24
Without cannulas	1.2 \pm 0.4	2.2 \pm 0.9	3.1 \pm 0.9	3.2 \pm 1.0
With cannulas	1.8 \pm 0.5	2.1 \pm 0.6	2.3 \pm 0.5	2.4 \pm 0.5

^a Unpaired t-test showed that, for either interval of time post-dosing, the differences in mean values between rats without or with biliary cannulas were not statistically significant; $P > 0.05$.

Table 3

Urinary excretion of 1-OHP following intravenous administration of 5 $\mu\text{mol/kg}$ of pyrene in non cannulated rats supplied with ordinary water, sweetened water or sweetened water with added electrolytes

Drinking water content	Cumulative 1-OHP excretion (percentage of dose) mean \pm S.D. for 4 rats ^a			
	Time interval post-dosing (h)			
	0-6	0-12	0-18	0-24
Ordinary water	0.3 \pm 0.1	0.5 \pm 0.2	0.6 \pm 0.2	0.7 \pm 0.3
Sweetened water ^b	1.1 \pm 0.3	2.0 \pm 0.6	2.7 \pm 0.6	2.9 \pm 0.6
Sweetened water with added electrolytes ^c	1.2 \pm 0.6	2.1 \pm 1.2	2.9 \pm 1.4	3.0 \pm 1.5

^a Unpaired t-test showed that, for each interval of time post-dosing, mean 1-OHP excretion in rats drinking ordinary water was statistically different from the values obtained in rats drinking sweetened water or sweetened water with added electrolytes with $P < 0.05$. Differences in mean excretion values between these two latter groups were not statistically significant; $P > 0.05$.

^b Drinking water containing glucose (40 g/l) and saccharin (1.5 g/l).

^c Drinking water containing glucose (40 g/l), saccharin (1.5 g/l) and electrolytes (NaCl 0.9% and KCl 0.05%).

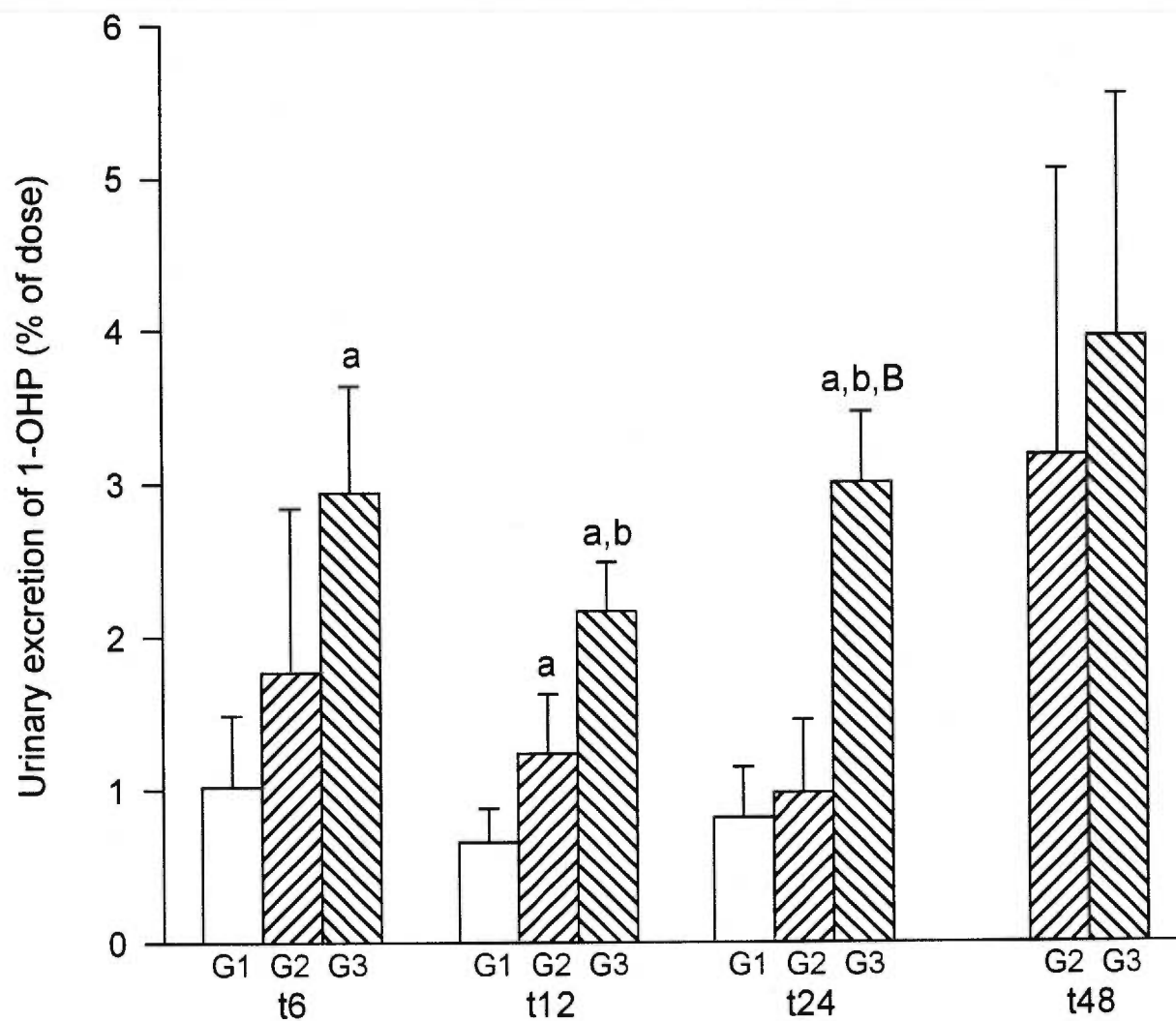


Fig. 4. Percentage of pyrene dose excreted in urine as 1-OHP over a 24-h period following intravenous administration of 5 $\mu\text{mol/kg}$ of pyrene in non cannulated rats provided with ordinary water (G1), sweetened water (G2) or sweetened water with added electrolytes (G3) and for which food was removed 6 (t6), 12 (t12), 24 (t24) and 48 (t48) h prior to dosing (mean \pm S.D. for 4 rats per group).

a: statistically different than G1

b: statistically different than G2

B: statistically different than comparable group at t12

4. Discussion

This study was designed to investigate the effect of the exposure route and the administered dose on the urinary and biliary excretion of 1-OHP in rats. It was intended to complete our knowledge on the excretion kinetics of 1-OHP and to further verify that 1-OHP in urine is a potentially useful bioindicator of PAH exposure.

It was shown that, whatever the exposure route and the administered dose, excretion kinetics of 1-OHP in bile resembled those depicted in urine. It was also determined that both urinary and biliary excretion kinetics of intravenously or orally administered pyrene were similar at all dose levels and a good relationship between pyrene dose and amounts of 1-OHP excreted was observed. Thus, the exposure dose had no significant effect on the mean percentage of administered pyrene excreted as 1-OHP in both urine and bile after 6, 12, 18, and 24 h post-dosing. These findings suggest that phase I biotransformation enzymes implicated in the formation of 1-OHP and phase II conjugating enzymes were not saturated over the range of doses tested. It also appears that renal and biliary mechanisms of excretion implicated in the elimination of 1-OHP were not saturated. Indeed, 1-OHP is excreted mostly in the conjugated form, hence mainly as sulfo-conjugates in rat urine and glucurono-conjugates in bile and feces (Boylard and Sims, 1964). In the kidney, in addition to being eliminated by glomerular filtration, a significant part should be cleared by tubular secretion (Friis, 1991). Glucurono- and sulfo- conjugates are actively transported by the para-aminohippurate system into the tubular lumen (Bessegir and Roch-Ramel, 1987; Ullrich and Rumrich, 1993). Possible saturation of this transport system could thus be anticipated. On the other hand, biliary excretion of organic

anions is also due to an active transport system (Sathirakul et al., 1994; Klaassen and Watkins, 1994). Since a large part of 1-OHP produced *in vivo* is excreted via the bile, it would have been likely that a saturation of biliary transport of 1-OHP would have resulted in an increased urinary excretion and consequently lower proportions recovered in bile.

Data from Withey et al. (1991) also indicated that increasing doses of pyrene produced a constant mean percentage of total ^{14}C recovered in urine after intravenous and oral ^{14}C -pyrene administration. While a good relationship between dose and fecal excretion of ^{14}C -pyrene was depicted following intravenous injection, a decrease in fecal excretion of ^{14}C -pyrene with increasing dose was apparent following oral administration. These authors however determined that bioavailability of orally administered ^{14}C -pyrene doses ranged between 73 and 84% ensuring an effective absorption from the gastrointestinal tract. On the other hand, they also reported that recovery of ^{14}C -pyrene in bile decreased with increasing intravenous and oral doses. It should be stressed that in this case, total ^{14}C -pyrene was measured. Results depicted could then have reflected a saturation of excretion of major metabolites other than 1-OHP such as glutathione, cysteinylglycine and cysteine conjugates which originate from pyrene-4,5-epoxide or such as glucurono-conjugates of pyrene-4,5-dihydrodiol (Boyland and Sims, 1964; Klaassen and Watkins, 1994). Saturation of the conjugation pathways leading to the formation of glutathione conjugates and their corresponding cysteinylglycine and cysteine derivatives is also possible (Sipes and Gandolfi, 1991). Kotin et al. (1959) have also shown that excretion of radiolabeled BaP metabolites in bile increased proportionally with the intravenously administered dose until a saturation point occurred which corresponded to a dose of approximately $3.2 \mu\text{mol/kg}$ of ^{14}C -BaP. As in the study of Withey et al. (1991), individual metabolites were not

quantified. Nonetheless, it appears that saturation of biliary excretion of BaP and its metabolites at molar doses below those obtained with pyrene is not unlikely. Indeed, in several reports, administration of labeled BaP and pyrene have allowed to determine that biliary and thus fecal excretion of labeled BaP was quantitatively more important than that of labeled pyrene (Chipman et al., 1983; Camus et al., 1984; Withey et al., 1991, 1993b, 1994). This is presumably linked to the higher molecular weight of conjugated BaP metabolites than conjugated pyrene metabolites favoring a larger biliary excretion of the former.

Regarding exposure routes, results of the current study have shown that urinary and biliary excretion kinetics of 1-OHP following intravenous injection compared well with those determined following oral administration and that the administration route had no significant effect on the mean percentage of the pyrene dose excreted as 1-OHP in both urine and bile after 6, 12, 18, and 24 h post-dosing. In a previous study, it has also been determined that absolute and relative urinary excretion of two major BaP metabolites, namely 3-hydroxyBaP and BaP-4,5-dihydrodiol along with the time course of their excretion in urine were similar by these two administration routes (Bouchard and Viau, 1997). As it appeared then for BaP metabolites, results presented in the current report also suggest that pyrene is very efficiently absorbed by the gastrointestinal tract, that the major biotransformation site would appear to be the liver following intravenous and oral administration of pyrene and that tissue distribution proceeds similarly in both cases. Using the data from Withey et al. (1991), simulation of pyrene pharmacokinetics following intravenous and oral administration have been described using a physiologically-based pharmacokinetic model (Haddad et al., 1998a). Oral absorption was represented by an input into the liver and was described as a first order process. By comparing the

simulated and experimental data, it however appeared that absorption was less efficient at a dose of 15 mg/kg (74.2 $\mu\text{mol/kg}$) of pyrene as compared to doses of 2, 4, 6 and 9 mg/kg (9.9, 19.8, 29.7 and 44.5 $\mu\text{mol/kg}$). Furthermore, following both intravenous and oral administration of pyrene, metabolism was described in the liver only since in a previous *in vitro* study using rat lung post-mitochondrial fractions, it was determined that metabolic activity of lungs towards pyrene was negligible (Haddad et al., 1998b). In the model, tissue uptake and distribution as well as binding to proteins in liver and lungs were similar by these two routes.

Furthermore, it was also determined in the present report, that 1-OHP was excreted predominantly in bile following intravenous and oral administration of pyrene. On the average, $2.7 \pm 0.8\%$ of the pyrene intravenous and oral doses was excreted as 1-OHP in urine ($n= 40$) and $8.9 \pm 2.3\%$ was found in bile ($n= 40$) over the 24-h period following treatment. The small percentage excreted as 1-OHP can in part be explained by the fact that some part of the dose remains in the organism after 24 h. With the physiologically-based pharmacokinetic model for pyrene (Haddad et al., 1998a), it can be predicted that, following intravenous administration of 50 $\mu\text{mol/kg}$ of pyrene, 88.25% of the dose will be metabolized after 24 h and 11.75% will remain in the body as unchanged pyrene at that time. The disposition of the metabolites cannot however be established with this model. These findings further suggest that unaccounted metabolites other than 1-OHP as measured in this study represent a large fraction of the excreted dose. Although 1-OHP has been shown to be the major metabolite produced *in vitro* using rat post-mitochondrial liver fractions or microsomes, other metabolites are formed (Sims, 1970; Grover et al., 1972; Jacob et al., 1982). Biotransformation products of 1-OHP have been identified, namely 1,6- and 1,8-dihydroxypyrene but appear to be present in very small

amounts. Another major metabolite produced is pyrene-4,5-dihydrodiol which originates from the formation of pyrene-4,5-epoxide (Grover et al., 1972). Results from the *in vitro* experiment conducted by Jacob et al. (1982) using liver microsomes from non-induced rats indicated that, about 29% of the amount of pyrene incubated 30 min with liver microsomal proteins formed metabolites (i.e. 16% of pyrene formed 1-OHP whereas 12.3% was biotransformed as other metabolites) and 71% remained as unchanged pyrene. 1-OHP represented 57% of the total amount of metabolites, pyrene-4,5-dihydrodiol accounted for 21%, an unidentified diphenol for 6%, 1,6-dihydroxypyrene for 11% and an unidentified triol for 5%. Hence, of the total amount of pyrene metabolized, 43% corresponded to metabolites other than 1-OHP.

In vivo, oxidation at the 4,5-position appears to lead to the formation of mercapturic acids excreted in urine and bile and cysteine, cysteinylglycine and glutathione conjugates recovered in bile (Boyland and Sims, 1964). Although glutathione conjugates and their derivatives are formed in *in vitro* experiments using liver homogenates, they were not measured in the previously mentioned studies. On the other hand, in microsomal fractions, conjugating enzymes are absent. Boyland and Sims (1964) have identified pyrene metabolites found in bile and urine of rats. In urine, sulfo- and glucurono-conjugates of 1-OHP, of 1,6- and 1,8-dihydroxypyrene and of pyrene-4,5-dihydrodiol together with some N-acetyl-S-(4,5-dihydro-4-hydroxy-5-pyrenyl)-L-cysteine were identified. In bile, N-acetyl-S-(4,5-dihydro-4-hydroxy-5-pyrenyl)-L-cysteine and the corresponding cysteine, cysteinylglycine and glutathione conjugates have been detected along with the glucurono-conjugates of pyrene-4,5-dihydrodiol and 1-OHP. The mercapturic acids and the cysteine, cysteinylglycine and glutathione conjugates could not be detected with the method used in the current report and, 1,6- and

1,8-dihydroxypyrene and pyrene-4,5-dihydrodiol were not quantified. The sum of these urinary and biliary metabolites could however have accounted for a significant fraction of the excreted dose. In the case of BaP, it has been reported that following intravenous administration of 3 $\mu\text{mol/kg}$ of ^{14}C -BaP, only 40% of the ^{14}C found in the bile was extractable into ethyl acetate after incubation with β -glucuronidase and arylsulfatase and that the fraction remaining in the aqueous phase was probably glutathione and other thioether conjugates (Chipman et al., 1981).

Withey et al. (1991) reported that 22 to 40% and 21 to 52% of the ^{14}C -pyrene dose was recovered in urine and feces, respectively, 24 h post-dosing. It should be pointed out that total ^{14}C -pyrene was determined and that metabolites other than 1-OHP as measured in the current study were therefore accounted for. In accordance with our results, Jongeneelen et al. (1985) reported that 1 to 2% of pyrene dose was excreted in rat urine as 1-OHP. Jacob et al. (1989) also reported that a very small percentage of pyrene dose was excreted in rat urine as 1-OHP: 0.4% after intraperitoneal and oral administration, 0.3% after intratracheal instillation and 0.01% after intrapulmonary instillation. They also showed that 1-OHP represented more than 85% of what was excreted as unchanged pyrene and 1-OHP in urine and feces after oral pyrene administration indicating that pyrene is excreted mostly in the metabolized form. Surprisingly, in feces, mean percentage of the pyrene dose excreted as 1-OHP over the 24-h period following oral administration (42%) was markedly different from that obtained after intraperitoneal injection (0.8%). These authors commented that with the treatment and gas chromatography method used in their study, 1- and 4-methoxypyrene were not distinguishable and therefore that in addition to 1-OHP, excretion products such as pyrene-4,5-dihydrodiol which form 4-hydroxypyrene by acidic saponification or 4-pyrenylmercapturic acid which yields

4-hydroxypyrene after alkaline saponification could be present.

On the other hand, as mentioned previously, the average percentage of pyrene dose excreted as 1-OHP in urine was 2.7% in the present study. In previous experiments conducted in our laboratory, a value of 0.7% was however obtained (Bouchard and Viau, 1996; Bouchard and Viau, submitted). Discrepancies in the results were quite intriguing. It was first thought that cannulation could have modified 1-OHP urinary excretion. It was however determined that 1-OHP urinary excretion values were similar in rats without and with biliary cannulas. It should be mentioned in passing that these findings also suggest that enterohepatic recirculation does not appear to affect 1-OHP urinary excretion appreciably. The only differences between the current study and the previous ones were the supplying of food and water. In the previous reports, rats were given sweetened drinking water during all the course of the experiment, and food which was removed 24 h prior to dosing. In the current study, rats were given sweetened drinking water and, following cannulation, sweetened water with added electrolytes. Food was provided until 6 h before treatment. It was therefore verified if the addition of sugar or sugar plus electrolytes to the drinking water had an effect on 1-OHP urinary excretion in non cannulated rats. Interestingly, providing sweetened drinking water or sweetened water with added electrolytes to rats for which food was removed 6 h prior to dosing resulted in similar excretion profiles and amounts excreted were almost identical. In these cases, 1-OHP urinary excretion was however increased by approximately four times compared to rats drinking tap water only although the excretion profile did not appear to be different. Furthermore, in rats drinking ordinary water, molar fraction of pyrene dose recovered as 1-OHP in urine was 0.7% which corresponds to what was obtained in the other reports (Bouchard and Viau, 1996; Bouchard and

Viau, submitted).

The urinary excretion of 1-OHP was consequently measured in non cannulated rats provided with either normal water, sweetened water or sweetened water with added electrolytes and for which food was removed 6, 12, 24 or 48 h prior to dosing. This allowed to observe that time of food-fasting did not influence 1-OHP excretion in rats drinking ordinary water or sweetened water supplemented with electrolytes contrary to rats drinking sweetened water. Furthermore, rats given sweetened water with added electrolytes had higher excretion values than rats drinking ordinary water. On the other hand, in rats supplied with sweetened water, when food was removed 6 and 48 h prior to dosing, 1-OHP excretion values were similar to those of rats given sweetened water with added electrolytes and when food was removed 24 h before treatment, values approached those obtained in rats drinking tap water only. A priori, it would appear that in rats deprived of food, giving sweetened water with added electrolytes is closer to normal physiological conditions than providing tap water only. Post-surgical administration of drinking water supplemented with glucose, sodium chloride and potassium chloride has indeed been recommended to aid recovery and limit weight loss by providing a caloric and electrolyte source and by maintaining hydration (Colwell, 1950; Rath and Hutchison, 1989; Heitmeyer and Powers, 1992).

In conclusion, the linear dose-excretion relationship for 1-OHP in urine or bile following either intravenous or oral administration of pyrene further justifies the use of this metabolite in urine as a bioindicator of PAH exposure. The similarities in both 1-OHP urinary and biliary excretion following intravenous and oral administration suggests that oral absorption is very efficient over the range of

doses tested and that the determinants of distribution, metabolism and excretion are common following exposure by these two routes. Furthermore, although biliary excretion of 1-OHP was more important than its urinary excretion, total 1-OHP excretion over 24 h represented a small percentage of the pyrene dose. These findings, when compared with the simulated values from a physiologically-based pharmacokinetic model for pyrene, suggest that some part of the pyrene dose remains in the organism after 24 h and that unaccounted metabolites other than 1-OHP as measured in the current study represent a significant proportion of total metabolites excreted in urine and bile.

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ARTICLE 4

Urinary excretion kinetics of 1-hydroxypyrene following intravenous administration of binary and ternary mixtures of polycyclic aromatic hydrocarbons in the rat

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Abstract

The effect of exposure to binary and ternary mixtures of polycyclic aromatic hydrocarbons (PAHs) on the urinary excretion kinetics of 1-hydroxypyrene (1-OHP) has been examined. Male Sprague-Dawley rats were administered intravenously 5 $\mu\text{mol/kg}$ of pyrene alone or in combination with 0.5, 5 or 25 $\mu\text{mol/kg}$ of either naphthalene, benzo(a)pyrene (BaP), or both. Urine samples were collected at frequent intervals over 48 h. The kinetics of 1-OHP in urine was not altered by the presence of either naphthalene or BaP in the mixtures, at least from 4 h post-dosing. Indeed, none of the injected mixtures significantly modified the first order apparent elimination half-life of 1-OHP in urine obtained for the 12 to 42-h period where mean values ranged between 6.2 and 9.6 h. However, while the presence of naphthalene or the low BaP dose of 0.5 $\mu\text{mol/kg}$ in the mixtures did not have a significant effect on the total excretion of 1-OHP, BaP doses of 5 and 25 $\mu\text{mol/kg}$ in the mixtures significantly increased the amount of 1-OHP excreted in urine. Mean percentages of the pyrene dose excreted as 1-OHP after injection of pyrene in combination with 0.5, 5 and 25 $\mu\text{mol/kg}$ BaP were respectively increased by 1.3, 2.2 and 2.6 times compared to the value obtained after administration of pyrene alone. The percentages determined after concomitant administration of pyrene and 0.5, 5 and 25 $\mu\text{mol/kg}$ of BaP plus naphthalene were 1.4, 1.8 and 2.4 times, respectively, the value obtained after administration of pyrene singly. The observed effect of BaP (5 or 25 $\mu\text{mol/kg}$) on 1-OHP total excretion appears to result from BaP enzyme induction of pyrene metabolism. Lack of effect of naphthalene appears to be due to its weak P450 1A1 enzyme induction capacity. Absence of significant effect of the low BaP dose in the mixtures (0.5 $\mu\text{mol/kg}$) suggests that 1-OHP in urine is useful as a bioindicator of occupational and environmental exposures to PAH mixtures.

Key words 1-Hydroxypyrene · Urinary excretion kinetics · Chemical mixtures · Polycyclic aromatic hydrocarbons · Pyrene

Introduction

Polycyclic aromatic hydrocarbons (PAHs) are a broad class of ubiquitous environmental contaminants. They are present as mixtures in coal tar and coal tar-derived products, urban and industrial air, food, water, soil and sediments (Lindstedt and Sollenberg 1982; Nikolaou et al. 1984; Rogge et al. 1991; Lee et al. 1995; Wild and Jones 1995). Over 500 airborne PAHs have been detected and over 300 have been identified in coal tar (Bjorseth 1983). However, 16 PAHs, which often account for 80 to 90% of the sum of all PAHs present in mixtures, have been classified as priority substances by the U.S. Environmental Protection Agency (U.S. EPA 1979). These include pyrene, benzo(a)pyrene (BaP) and naphthalene.

Several years ago, Jongeneelen et al. (1987) proposed the use of urinary 1-hydroxypyrene (1-OHP) as a bioindicator of exposure to PAH mixtures. This however raises questions concerning the possible influence of concurrent exposure to many PAHs on the excretion of 1-OHP in urine. Since the various individual PAHs are likely to be biotransformed by the same P450 isoenzymes (Jacob et al. 1982; Conney et al. 1994), alteration of the metabolism of one PAH by another member of this class in a given mixture may occur as a result of enzymatic induction or competition for enzymatic or non-enzymatic binding sites (Krishnan et al. 1994).

It should also be stressed that biliary transport of anions, which is due to an active transport system, is a saturable process that is prone to competitive inhibition (Sathirakul et al. 1994). Most PAHs form monohydroxylated metabolites, the glucurono-conjugates of which have molecular weights spreading

on both sides of the approximate threshold value of 325 in the rat (e.g. glucurono-conjugates of 1-hydroxynaphthalene: 305, of 1-OHP: 378 and of 3-hydroxyBaP: 428) (Klaassen and Rozman 1991). Therefore, there is a possibility for competitive inhibition of the biliary transport of conjugated 1-OHP by conjugated metabolites of other PAHs. Some researchers have examined the urinary excretion of specific PAH metabolites after exposure to PAH mixtures in controlled conditions, in general, creosote and coal tar exposures in rats (Jongeneelen et al. 1986), in psoriatic patients (Jongeneelen et al. 1985; Jongeneelen et al. 1986; Clonfero et al. 1989; Hansen et al. 1993) or human volunteers (van Rooij et al. 1993; Van Maanen et al. 1994; Kang et al. 1995; Viau and Vyskocil 1995). However, these studies were not conducted in parallel with studies on individual compounds to systematically evaluate the effect of mixed PAH exposures on the urinary excretion of specific metabolites.

The objective of the present study was therefore to assess the effect of exposure to simple binary (pyrene and naphthalene or pyrene and BaP) and ternary mixtures of PAHs (pyrene, naphthalene and BaP) on the urinary excretion of 1-OHP in the rat.

Materials and methods

Chemicals

Pyrene (99% purity) and naphthalene (>99% purity) were purchased from Aldrich Chemical Co. (Mississauga, ON, Canada) and used as received. BaP (approximately 98% purity) was purchased from Sigma Chemical Co. (St Louis, MO) and used as received. Reference standard of 1-OHP (>99% purity) was obtained from the NCI Chemical Carcinogen Reference Standards distributed by Midwest Research Institute (Kansas, MO) and used as received. β -glucuronidase/arylsulfatase (100 000 Fishman U/ml and 800 000 Roy U/ml from *Helix pomatia*) was purchased from Boehringer Mannheim GmbH (Laval, Quebec, Canada). Alkamuls EL-620 (formerly Emulphor 620) was kindly provided by Rhone-Poulenc (St-Hyacinthe, Quebec, Canada). HPLC-grade methanol was obtained from Caledon (Georgetown, ON, Canada). Ascorbic acid was purchased from J.T. Baker Chemical Co. (Phillipsburg, NJ).

Animals

Male Sprague-Dawley rats (Charles River Canada Inc., St-Constant, Quebec, Canada) weighing 200 to 250 g were used. The animals were kept in individual metabolic cages. Lighting was maintained on a 12-h light-dark cycle and room temperature was kept at $22 \pm 3^\circ\text{C}$. Animals were cared for in accordance with the principles and guidelines of the Canadian Council on Animal Care.

Animal treatment

Groups of four to six rats each were administered intravenously 5 $\mu\text{mol/kg}$ of pyrene alone or in combination with either naphthalene (0.5, 5 or 25 $\mu\text{mol/kg}$), BaP (0.5, 5 or 25 $\mu\text{mol/kg}$) or both (0.5, 5 or 25 $\mu\text{mol/kg}$ of each of naphthalene and BaP) by a tail vein. Pyrene, naphthalene and BaP were dissolved in 20% emulphor: 80% isotonic glucose solutions. One ml of solution was injected per kg of body weight when administering pyrene (5 $\mu\text{mol/kg}$) in combination with 0.5 or 5 $\mu\text{mol/kg}$ of naphthalene and/or BaP. Three ml of solution was injected per kg when administering pyrene (5 $\mu\text{mol/kg}$) in combination with 25 $\mu\text{mol/kg}$ of naphthalene and/or BaP to ensure complete solubilization of BaP. When injecting pyrene singly, 4 rats were given 1 ml of solution per kg and 2 rats were given 3 ml of solution per kg. This confirmed that the amount of vehicle injected did not influence the urinary excretion of 1-OHP (data not shown).

Urine sampling

Urine samples were collected at frequent intervals over a 48-h period following injection ($t= 2, 4, 6, 8, 10, 12, 18, 24, 30, 42, 48$ h) to establish the detailed kinetic profile of 1-OHP. Rats received drinking water containing glucose (40 g/l) and saccharin (1.5 g/l) causing a polydipsic behavior and, consequently, a physiologic polyuria thus allowing for frequent urine collections (Chouinard and Viau 1992). Control urine samples were collected during the 2-h period preceding injection.

Urine analysis

Urine samples were collected over thymol and frozen at -20°C until analysis. Samples were treated using the method described by Jongeneelen et al. (1987). Efficiency of the extraction of hydrolyzed

metabolites was determined using untreated rat urine samples spiked with 12.5, 25, 50 and 100 pmol of authentic 1-OHP reference standard. Recovery from 20 spiked samples was $65 \pm 2\%$ (mean percentage \pm SE). Results presented in this article have been corrected for recovery.

Samples were analyzed by a high performance liquid chromatographic system composed of an automatic injector AS-100 (Bio-Rad, Richmond, CA), a binary pump model 250 (Perkin-Elmer, Buckingham, England), a LC-18 Supelcosil 250×4.6 mm column (Supelco, Oakville, ON, Canada), a fluorescence detector LS-40 (Perkin-Elmer), a PE Nelson 900 interface (Perkin-Elmer) and a PE Nelson turbochrom 3 integration system. The column temperature was set at 32°C . Excitation and emission wavelengths were 242 and 388 nm, respectively. Under these conditions, separation was performed in 25 min using 71% methanol: 29% water mobile phase at a flow rate of 0.8 ml/min. Recovery and reproducibility of the analyte were improved by the addition of ascorbic acid (1 mg/l) to the methanol eluent (Bouchard et al. 1994). Injection volume was 20 μl .

Statistical analysis

Influence of exposure scenarios (administration of pyrene alone or in combination with naphthalene and/or BaP) on 1-OHP excretion values at each time-point of the excretion curves, on 1-OHP elimination half-life and on the percentage of pyrene dose excreted in urine as 1-OHP over 48 h was assessed using one-way analysis of variance; $P = 0.05$ was considered as the level of significance. In the case of a significant difference among the group means, Bonferroni post-test was performed to determine which values obtained after mixed exposures were significantly different from the value obtained after injection of pyrene alone.

Results

Urinary excretion of 1-OHP following intravenous administration of pyrene singly or in combination with naphthalene

Figure 1 shows the effect co-administration of naphthalene (0.5, 5 or 25 $\mu\text{mol/kg}$) and pyrene (5 $\mu\text{mol/kg}$) on the urinary excretion kinetics of 1-OHP. Regardless of the dose, naphthalene in the mixtures did not alter the kinetics of 1-OHP in urine nor its quantitative excretion. Careful examination of the excretion time curves showed two apparent phases of elimination, a more rapid phase followed by a slower phase. Data in Table 1 show that first order apparent half-lives of 1-OHP calculated for the 12 to 42-h period following administration of 5 $\mu\text{mol/kg}$ of pyrene singly or in combination with 0.5, 5 or 25 $\mu\text{mol/kg}$ of naphthalene were not significantly different. It is also noteworthy that excretion levels of 1-OHP between 42 and 48 h had reached background values of untreated rats. Furthermore, on average, 92 to 95% of the amount of 1-OHP excreted in urine over the 48-h collection period was eliminated during the first 24 h in all treatment groups. The percentages of the pyrene dose excreted as 1-OHP over the 48-h urine collection period following injection of the binary mixtures of naphthalene and pyrene were not statistically different from the value obtained after administration of pyrene singly (Table 1).

Urinary excretion of 1-OHP following intravenous administration of pyrene singly or in combination with BaP

Figure 2 shows the effect of intravenously administered binary mixtures of BaP (0.5, 5 or 25 $\mu\text{mol/kg}$) and pyrene (5 $\mu\text{mol/kg}$) on the urinary excretion kinetics of 1-OHP. For the 2 to 48-h period, BaP doses of 5 and 25 $\mu\text{mol/kg}$ and not 0.5 $\mu\text{mol/kg}$ in the mixtures increased significantly the amounts of 1-OHP excreted in urine as compared to those obtained after injecting pyrene singly. Although maximum excretion of 1-OHP was observed during the first sampling period (0-2 h) after administration of pyrene alone, and between 2 to 4 h following administration of the pyrene and BaP mixtures, the kinetics of 1-OHP in urine for the 4 to 48-h period post-dosing did not appear to be influenced by the presence of BaP in the mixtures. In fact, as shown in Table 1, BaP (0.5, 5 and 25 $\mu\text{mol/kg}$) did not significantly modify the first order elimination half life of 1-OHP calculated for the 12 to 42-h period. On the other hand, the percentages of pyrene dose excreted as 1-OHP in urine over the 48-h collection period following injection of binary mixtures of 5 $\mu\text{mol/kg}$ of pyrene and 0.5, 5 or 25 $\mu\text{mol/kg}$ of BaP were respectively increased by 1.3, 2.2 and 2.6 times as compared to the value obtained after administration of pyrene alone (Table 1). To further emphasize the lack of BaP effect on 1-OHP excretion kinetics, the standardized urinary excretion kinetics of 1-OHP were also represented where excretion rates were expressed as a percentage of total 1-OHP recovered in urine over the 48-h collection period (Fig. 3). It can be seen that, at least from 4 h post-dosing, excretion rates of 1-OHP were not altered by the presence of BaP in the mixtures. Moreover, following administration of pyrene and BaP mixtures, 90 to 94% of the amount of 1-OHP excreted in urine over the 48-h collection period was eliminated during the first 24 h. These values were similar to the value

of 93% observed after administration of pyrene alone.

Urinary excretion of 1-OHP following intravenous administration of pyrene singly or in combination with both naphthalene and BaP

Figure 4 shows the effect of administration of ternary mixtures containing pyrene (5 $\mu\text{mol/kg}$), and naphthalene plus BaP (0.5, 5 or 25 $\mu\text{mol/kg}$ each) on the urinary excretion kinetics of 1-OHP. As observed after administration of the binary mixtures of pyrene and BaP or naphthalene, the urinary excretion kinetics of 1-OHP did not seem to be altered when pyrene was administered in mixtures containing both naphthalene and BaP at least for the 4 to 48-h period. For the 12 to 42-h period post-dosing, elimination half-lives of 1-OHP in urine after mixed exposures were not significantly different from the value of 6.5 ± 0.5 h (mean \pm SE) obtained after administration of pyrene singly (Table 1). On the other hand, the amounts of 1-OHP excreted between 2 and 48 h post-dosing tended to be higher following the injection of pyrene in combination with 25 $\mu\text{mol/kg}$ of each of naphthalene and BaP than those recovered after administration of pyrene singly. Concomitant administration of pyrene and 0.5 or 5 $\mu\text{mol/kg}$ of each of naphthalene and BaP did not however have an effect on the amount of 1-OHP excreted in urine. The percentages of the pyrene dose excreted as 1-OHP over the 48-h urine collection period following injection of the ternary mixtures of 5 $\mu\text{mol/kg}$ of pyrene and 0.5, 5 or 25 $\mu\text{mol/kg}$ of both naphthalene and BaP were 1.4, 1.8 and 2.4 times, respectively, greater than the value observed after administration of pyrene singly (Table 1).

Thus, regardless of the dose, the presence of naphthalene and BaP in the binary or ternary mixtures

did not modify the urinary excretion kinetics of 1-OHP. However, BaP doses of 5 and 25 $\mu\text{mol/kg}$ and not 0.5 $\mu\text{mol/kg}$ in the mixtures caused a significant increase in the amount of 1-OHP excreted in urine (Table 1).

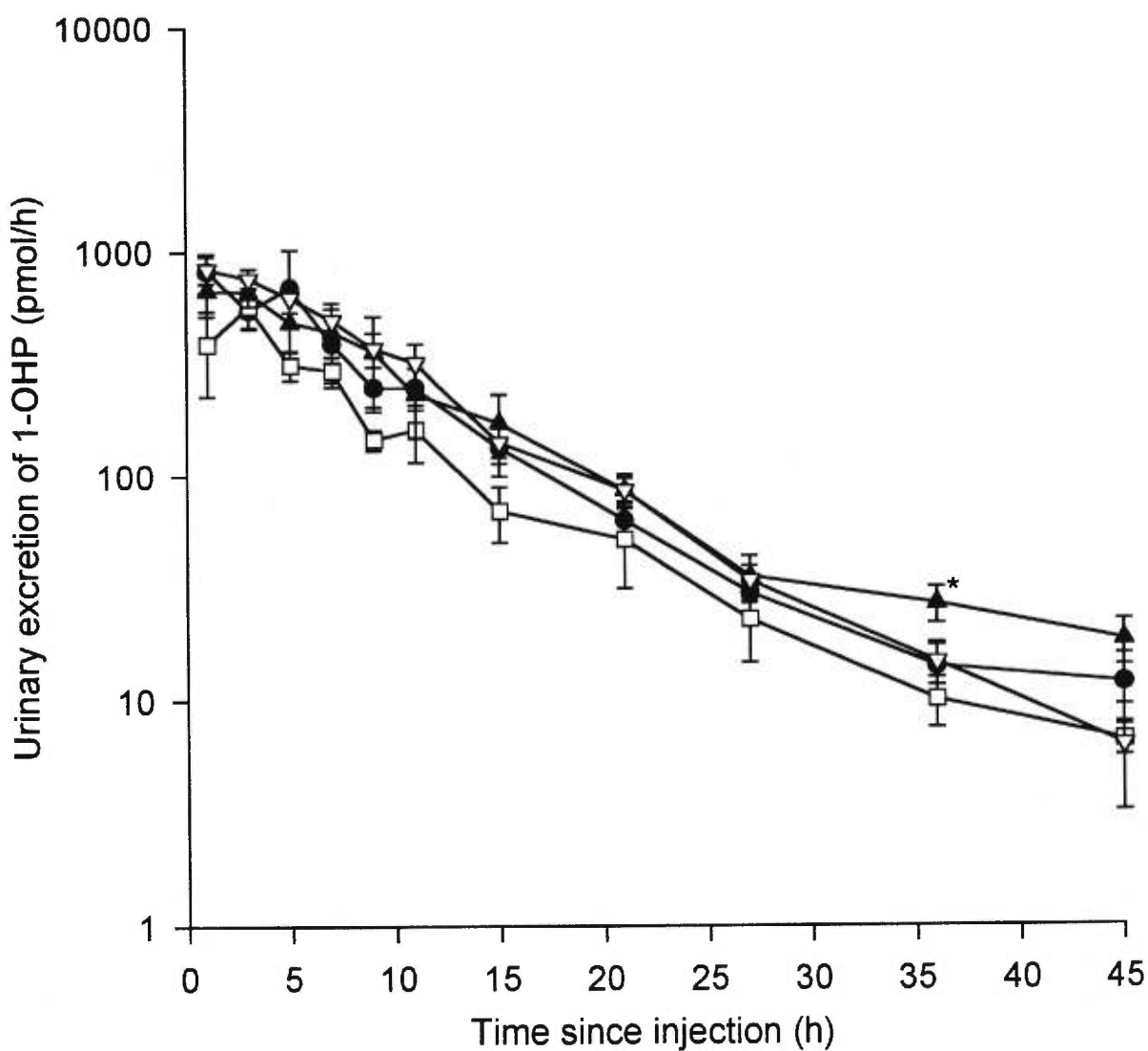


Fig. 1 Urinary excretion kinetics of 1-OHP following intravenous administration of 5 $\mu\text{mol/kg}$ of pyrene alone (\bullet) ($n = 6$) or in combination with 0.5 (\square), 5 (\blacktriangle), or 25 (∇) $\mu\text{mol/kg}$ of naphthalene ($n = 4$). Each point represents mean excretion rate and corresponds to the midpoint of the sample collection period and vertical bars are standard errors. * = Excretion rate significantly different from the value obtained after injection of pyrene alone with $P < 0.05$.

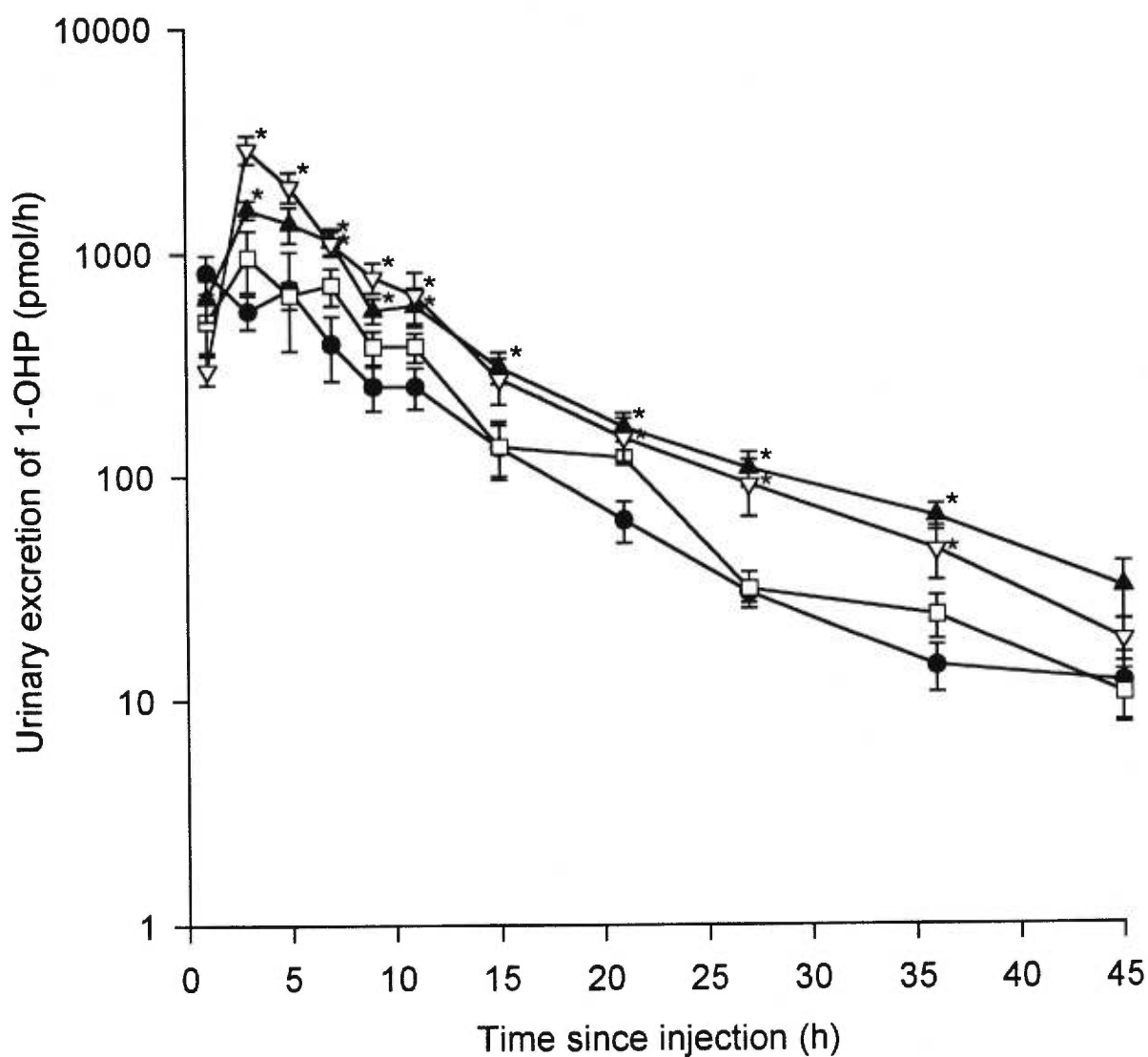
Table 1 First order apparent half-lives of 1-OHP and percentage of pyrene dose excreted in urine as 1-OHP over the 48-h period following intravenous administration of pyrene alone or in combination with naphthalene and/or benzo(a)pyrene

Pyrene dose ($\mu\text{mol/kg}$)	Naphthalene dose ($\mu\text{mol/kg}$)	BaP dose ($\mu\text{mol/kg}$)	Half-life (h) for the 12 to 42-h period ^a (mean \pm SE)	% of pyrene dose excreted in urine as 1-OHP ^b (mean \pm SE)
5	0	0	6.5 \pm 0.5	0.65 \pm 0.14
5	0.5	0	7.2 \pm 0.7	0.43 \pm 0.09
5	5	0	7.6 \pm 1.6	0.70 \pm 0.14
5	25	0	6.2 \pm 0.5	0.74 \pm 0.07
5	0	0.5	7.5 \pm 2.1	0.82 \pm 0.09
5	0	5	9.6 \pm 1.0	1.41 \pm 0.16*
5	0	25	8.4 \pm 0.4	1.71 \pm 0.27*
5	0.5	0.5	9.5 \pm 2.6	0.94 \pm 0.30
5	5	5	8.1 \pm 1.7	1.16 \pm 0.14
5	25	25	6.6 \pm 0.3	1.55 \pm 0.34*

^a ANOVA showed that the difference among the group means was not statistically significant.

^b ANOVA showed that the difference among the group means was statistically significant only for the experiments involving BaP. Post-test was performed to determine which values obtained after mixed exposures were significantly different from the value obtained after injection of pyrene alone.

* Significantly different from the value obtained after injection of pyrene alone with $P < 0.05$.

**Fig. 2**

Urinary excretion kinetics of 1-OHP following intravenous administration of 5 $\mu\text{mol/kg}$ of pyrene alone (●) ($n = 6$) or in combination with 0.5 (□), 5 (▲), or 25 (▽) $\mu\text{mol/kg}$ of benzo(a)pyrene ($n = 4$). Each point represents mean excretion rate and corresponds to the midpoint of the sample collection period and vertical bars are standard errors. * = Excretion rate significantly different from the value obtained after injection of pyrene alone with $P < 0.05$.

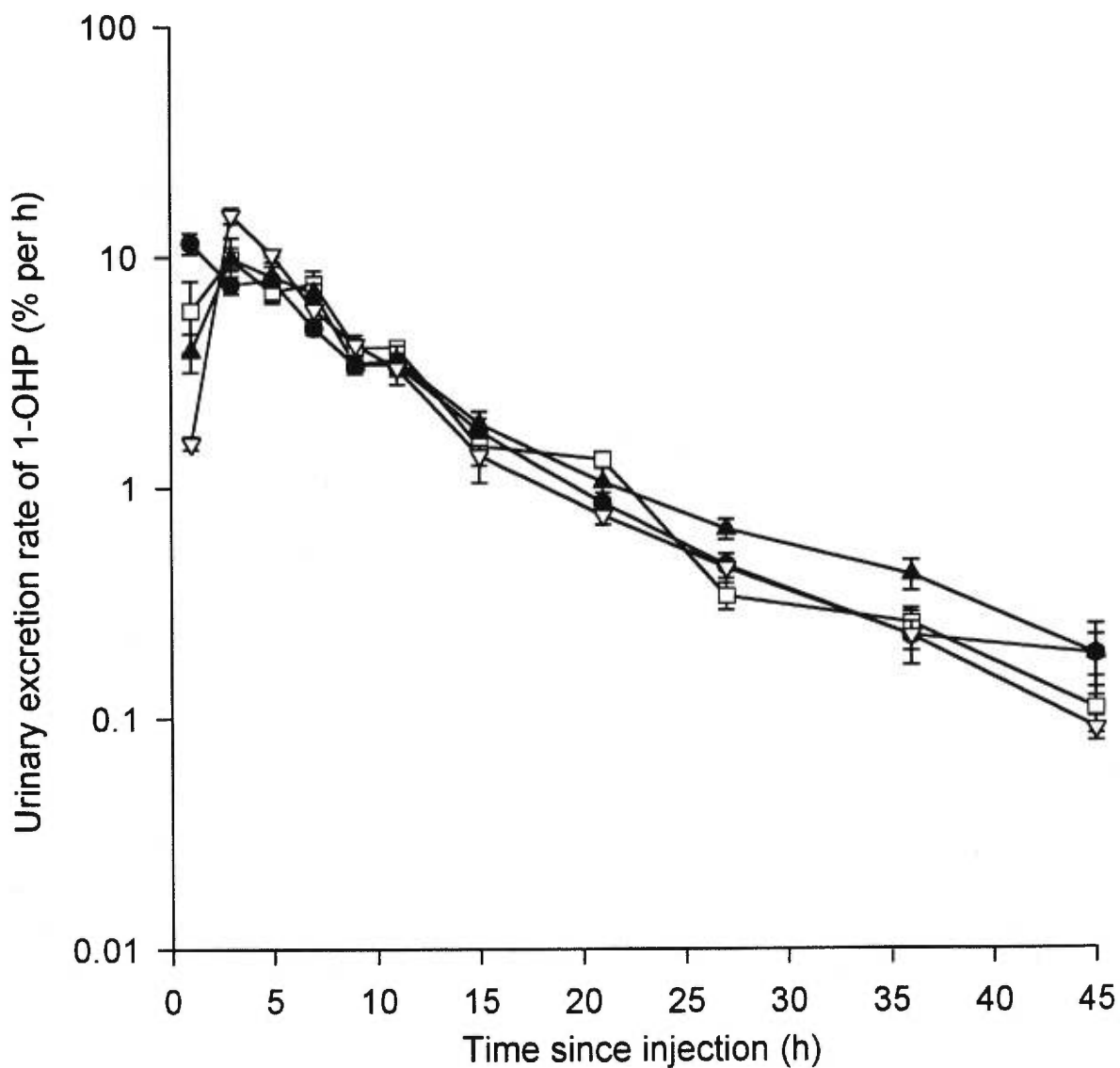


Fig. 3 Standardized urinary excretion kinetics of 1-OHP following intravenous administration of 5 $\mu\text{mol/kg}$ of pyrene alone (●) ($n = 6$) or in combination with 0.5 (□), 5 (▲), or 25 (▽) $\mu\text{mol/kg}$ of benzo(a)pyrene ($n = 4$), where 1-OHP excretion rates are expressed as a percentage of total amounts recovered in urine over the 48-h collection period.

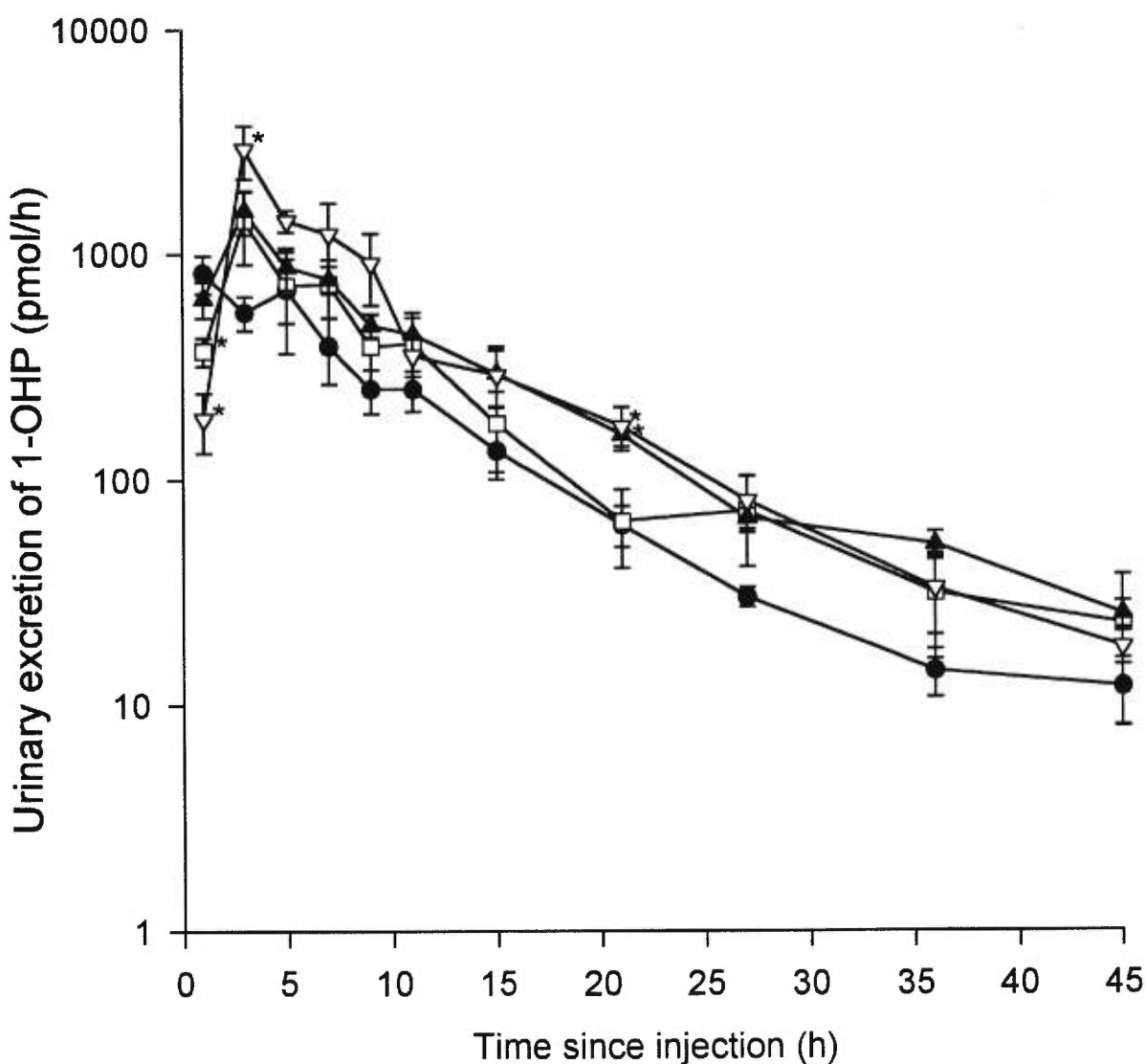


Fig. 4 Urinary excretion kinetics of 1-OHP following intravenous administration of 5 $\mu\text{mol/kg}$ of pyrene alone (●) ($n = 6$) or in combination with 0.5 (□), 5 (▲), or 25 (▽) $\mu\text{mol/kg}$ of both naphthalene and benzo(a)pyrene ($n = 4$). Each point represents mean excretion rate and corresponds to the midpoint of the sample collection period and vertical bars are standard errors. * = Excretion rate significantly different from the value obtained after injection of pyrene alone with $P < 0.05$.

Discussion

The increasing use of 1-OHP as a bioindicator of exposure to total PAHs requires a systematic evaluation of the urinary excretion of 1-OHP after exposure to pyrene alone or in combination with other PAHs. These contaminants are present as complex mixtures rendering the possible combinations extremely large even when considering only the 16 priority PAHs defined by the U.S. EPA (1979). The present study has evaluated the possible influence of “low” (2-ring compound) and “high” (5-ring compound) molecular weight PAHs on the urinary excretion of 1-OHP in the rat, in an effort to identify the potential interaction threshold dose of each of these PAHs.

Naphthalene, a non-carcinogenic PAH, was chosen as a low molecular weight PAH because: (1) it is abundant in environmental PAH mixtures (Bjorseth et al. 1978a,b, 1981), (2) it is a weak inducer of cytochrome P450 1A1 responsible for the biotransformation of PAHs (Ayrton et al. 1990) and (3) the molecular weight of the glucurono-conjugate of 1-hydroxynaphthalene (305), a major metabolite of naphthalene, lies below the threshold for biliary excretion in the rat (325) (Klaassen and Rozman 1991) suggesting that it would not interfere with the biliary transport of the glucurono-conjugate of 1-OHP. On the other hand, the probable human carcinogen BaP was chosen as a high molecular weight PAH because: (1) it is listed as one of the 16 priority PAHs (U.S. EPA 1979), (2) it is a strong P450 1A1 inducer (Conney et al. 1994) and (3) the molecular weight of the glucurono-conjugate of 3-hydroxyBaP (428), a major metabolite of BaP, is higher than the threshold for biliary excretion, hence a candidate for a possible competitive inhibition of glucurono-conjugate of 1-OHP biliary transport in the rat (Sathirakul et al. 1994).

Results showed that the presence of naphthalene and BaP in the mixtures did not alter the urinary excretion kinetics of 1-OHP, whatever the administered dose. The presence of naphthalene or 0.5 $\mu\text{mol/kg}$ BaP had no significant effect on the percentage of pyrene (5 $\mu\text{mol/kg}$) excreted as 1-OHP in urine. However, BaP doses of 5 and 25 $\mu\text{mol/kg}$ in the mixtures caused a significant increase the amount of 1-OHP excreted in urine. Recently, the detailed excretion profile of 1-OHP in the urine of volunteers exposed to creosote by the dermal route has been described (Viau and Vyskocil 1995). Visual examination of the profile suggested two phases and a half-life of 12 h was calculated for the 12 to 48-h period which corresponds to the value obtained after exposure to pyrene alone in the same volunteers (Viau et al. 1995). As observed in the current study, these latter values suggest that 1-OHP excretion profile is not influenced by a concomitant exposure to other PAHs at the concentration present in creosote.

The observed effect of 5 and 25 $\mu\text{mol/kg}$ BaP in the mixtures appears to result from the induction of P450-dependent-monoxygenase system (Conney 1982, 1994; Jacob et al. 1982; Moorthy et al. 1994). Jacob et al. (1982) reported that liver microsomes obtained from rats pretreated with BaP (40 mg/kg per day, for 3 days) and incubated with pyrene formed greater amounts of 1-OHP than the microsomes from control animals or those pretreated with a lower molecular weight PAH (phenanthrene, 40 mg/kg per day, for 3 days). Ioannides et al. (1984) showed that BaP induced P450 IA activity in liver, kidneys and lungs in contrast with the non-carcinogenic anthracene. Furthermore, the data published by Conney et al. (1957) suggest that a single intraperitoneal dose of 0.1 or 1 mg BaP in rats weighing between 40 and 60 g (i.e. doses of 6.6 to 9.9 $\mu\text{mol/kg}$ and 66 to 99 $\mu\text{mol/kg}$) caused two- to four-fold increase in BaP metabolism in the liver by 3 h post-dosing. These findings

are consistent with the possibility that enzyme induction occurs rapidly following BaP exposure and that, in the current study, the BaP doses which caused an increase in the amounts of 1-OHP excreted in urine are also likely to cause an induction of pyrene metabolism.

Unpublished results from our laboratory have shown that total 1-OHP excretion in urine increased linearly with dose in rats intravenously administered pyrene doses of 0.5 to 50 $\mu\text{mol/kg}$ and that the first order apparent elimination half-life of 1-OHP in urine is independent of dose. Therefore, it appears that the metabolic capacity is not saturated at the pyrene dose administered in the current study (5 $\mu\text{mol/kg}$). For poorly metabolized substances for which intrinsic metabolic clearance ($=V_{\text{max}}/K_m$ where V_{max} is the maximal velocity for metabolism and K_m represents the Michaelis affinity constant) is smaller than the hepatic blood flow rate (Q_H), enzyme induction can affect the rate of metabolism even at low exposure levels (Wang et al. 1997). In the case of pyrene, *in vivo* V_{max} value is 0.04 mg/kg/min and K_m value is 5.61 mg/l (Haddad 1997). The intrinsic clearance is 0.0027 l/min for a 250 g rat, a value smaller than the Q_H , 0.021 l/min (Krishnan and Andersen 1994). The corresponding hepatic extraction ratio ($(V_{\text{max}}/K_m)/(V_{\text{max}}/K_m + Q_H)$) is 0.114. These data suggest that pyrene is poorly metabolized to 1-OHP and that enzyme induction can increase pyrene metabolism at all dose levels. Enzyme induction will therefore increase the amounts of 1-OHP formed in the liver which will be reflected by an increase in the amounts of 1-OHP excreted in urine. Although increasing the rate of disappearance of unmetabolized pyrene in blood, induction will not affect the kinetics of 1-OHP in urine. Indeed, it has previously been reported that the excretion kinetics of 1-OHP in urine following administration of the parent compound or the metabolite were similar and that phase I biotransformation was not the rate-limiting step in the urinary excretion kinetics of 1-OHP (Bouchard

and Viau 1996). The urinary excretion kinetics of 1-OHP therefore reflects the elimination of the metabolite from the body and not the biotransformation of pyrene into 1-OHP.

A priori, competition for blood or tissue protein binding could be anticipated (Charlton et al. 1976; Muresan et al. 1987; Souès et al. 1989a,b; Busbee et al. 1990; Krishnan and Brodeur 1991). Displacement of protein-bound pyrene by BaP would render more pyrene available for biotransformation in the liver and hence increase the amounts of 1-OHP formed. However, it is not the Q_H that limits the extent of pyrene metabolism but rather the V_{max}/K_m . Therefore, increasing the circulating concentration of unchanged pyrene will not affect the amount of 1-OHP formed because metabolism is capacity-limited.

Another possible explanation for the observed effect of BaP containing mixtures could have been that conjugated BaP metabolites may compete for the biliary transport of conjugated 1-OHP (Guiney et al. 1978). This would cause an overflow of 1-OHP towards the plasma and thus an increase in its urinary excretion for a given constant dose of pyrene. This mechanism is however unlikely since competition for biliary excretion transport system is dependent on the concentration of competing conjugated BaP metabolites and should therefore be reflected by a change in the slope of 1-OHP excretion curves with time.

The lack of significant effect of 0.5 $\mu\text{mol/kg}$ BaP suggests that, in the context of low-dose occupational and environmental exposures, alteration of 1-OHP excretion by PAH mixtures is unlikely and that 1-OHP remains useful for the biological monitoring of global exposure to PAHs.

For example, using the threshold limit value (TLV) for exposure to PAHs in the workplace ($200 \mu\text{g}/\text{m}^3$ for coal tar pitch volatiles expressed as benzene soluble matter) and considering BaP represents approximately 5% of total PAH mixtures, the corresponding daily BaP dose to which workers would thus be exposed is $(200 \mu\text{g}/\text{m}^3 \times 10 \text{ m}^3/\text{working day} \div 70 \text{ kg}) \times 5\% \approx 1.4 \mu\text{g}/\text{kg}$ or $5.7 \text{ nmol}/\text{kg}$ which is 88 times lower than the low BaP dose used in the current study. If we consider allometry which is based on the assumption that physiological parameters vary in a regular manner across species and can be quantitatively described as a power function of the body weight (BW) (Krishnan and Andersen 1991), the $0.5 \mu\text{mol}/\text{kg}$ BaP dose used in the current study would correspond to a BaP human equivalent dose of $76 \text{ nmol}/\text{kg}$ using the equation $\text{dose}_{\text{human}} (\text{mass or mol}/\text{kg}) = \text{dose}_{\text{rat}} (\text{BW}_{\text{human}}/\text{BW}_{\text{rat}})^{-1/3}$, which is still 13 times higher than the BaP dose that would be observed at TLV. In comparison, considering that pyrene represents approximately 10% of PAH mixtures, the pyrene dose that would be observed at TLV is $3 \mu\text{g}/\text{kg}$ or $14 \text{ nmol}/\text{kg}$ which is 54 times lower than the pyrene human equivalent dose used in the current study ($764 \text{ nmol}/\text{kg}$). Of course, other PAHs than BaP also have an enzyme induction capacity but even when considering the worst exposure scenario which would be that the $200 \mu\text{g}/\text{m}^3$ threshold limit value would correspond entirely to BaP, a BaP dose of $28.6 \mu\text{g}/\text{kg}$ or $113 \text{ nmol}/\text{kg}$ in exposed workers can be calculated which is similar to the low BaP human equivalent dose of $76 \text{ nmol}/\text{kg}$ (or $19 \mu\text{g}/\text{kg}$) used in the present study causing no effect on 1-OHP urinary excretion.

In conclusion, the presence of naphthalene or BaP in the mixtures did not influence the urinary excretion kinetics of 1-OHP. However, BaP doses of 5 and $25 \mu\text{mol}/\text{kg}$ in the administered mixtures caused a significant increase in the percentage of administered pyrene excreted as 1-OHP in urine.

Provided that enzyme induction occurs within 2 to 4 h following exposure, BaP enzyme induction of pyrene metabolism appears to explain the increase in the amounts of 1-OHP excreted in urine following coexposure to BaP at doses of 5 $\mu\text{mol/kg}$ and above in the mixtures. The observed lack of significant mixture effect when administering pyrene in combination with naphthalene or the low BaP dose suggests that, in the context of low-dose occupational and environmental exposures, effect of mixtures on 1-OHP urinary excretion should be negligible, thus supporting the use of 1-OHP as a bioindicator of exposure to PAHs.

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ARTICLE 5

Kinetics of Tissue Distribution and Elimination of Pyrene and 1-Hydroxypyrene Following Intravenous Administration of ¹⁴C-Pyrene in Rats

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ABSTRACT

Kinetics of Tissue Distribution and Elimination of Pyrene and 1-Hydroxypyrene Following Intravenous Administration of ^{14}C -Pyrene in Rats. BOUCHARD, M., KRISHNAN, K., AND VIAU, C. (199X) *Toxicol. Sci.* XX, XX-XX.

The tissue distribution and elimination of pyrene and 1-hydroxypyrene (1-OHP) were evaluated in male Sprague-Dawley rats following an intravenous injection of 50 $\mu\text{mol/kg}$ of ^{14}C -pyrene. Blood and tissues were removed and urine and feces were collected at various time points up to 24 hr. ^{14}C -pyrene equivalents were measured by liquid scintillation counting, and pyrene and 1-OHP were analyzed by HPLC/fluorescence. At 1 hr, the largest fraction of the dose was found in adipose tissue, essentially as pyrene, and its elimination followed first order monophasic kinetics with a half-life ($t_{1/2}$) of 4.9 hr. In blood, liver, kidney, lung, muscle and gastrointestinal (GI) tract, kinetics of ^{14}C -pyrene equivalents was biphasic and average $t_{1/2}$ values for the terminal elimination phase (8 to 24 hr) ranged between 6.2 and 8.7 hr. Elimination of pyrene in blood and these tissues except the GI tract followed first order biphasic kinetics with average $t_{1/2}$ values of the terminal phase ranging between 3.6 and 5.4 hr. In the GI tract, a monophasic elimination kinetics of pyrene was observed with mean $t_{1/2}$ value of 3.1 hr. Kinetics of 1-OHP in blood and liver showed a monophasic elimination with mean $t_{1/2}$ values of 6.7 and 6.2 hr, respectively. Kinetics of 1-OHP in the other tissues was biphasic with average $t_{1/2}$ values of the terminal elimination phase ranging between 5.2 and 6.2 hr. At 24 hr, on average, 81.7% of the dose was recovered in the urine (57.2%), feces (18.3%) and GI tract (6.2%) as ^{14}C -pyrene

equivalents with 2.7 and 1.9% of dose excreted as 1-OHP in urine and feces, respectively. At all time points, 1-OHP in urine represented a constant fraction of total ^{14}C in urine and feces. These results indicate that (i) ^{14}C -pyrene was rapidly distributed, metabolized and eliminated from the body, and (ii) although 1-OHP represents a small percentage of total pyrene eliminated from the body, it remains a reliable indicator of systemic exposure to, and overall elimination of the ^{14}C associated with, this polycyclic aromatic hydrocarbon.

INTRODUCTION

Polycyclic aromatic hydrocarbons (PAHs) are highly lipophilic compounds formed mainly as a result of incomplete combustion or pyrolysis of organic materials (Harvey, 1997). Several PAHs and their mixtures are carcinogenic in humans (IARC, 1987; Boffetta *et al.*, 1997). The measurement of the urinary levels of the hydroxylated metabolite of pyrene, 1-hydroxypyrene (1-OHP), has been recommended for the evaluation of exposure to PAHs in the general and occupational environments (Levin, 1995; Holian, 1996). Despite the increasing use of urinary 1-OHP for the biological monitoring of exposure to PAHs (Omland *et al.*, 1996; Malkin *et al.*, 1996; Gilbert and Viau, 1997), the toxicokinetics of this metabolite remains poorly documented. A clear understanding of the kinetic relationship between exposure to the parent compound, pyrene, and elimination of 1-OHP is essential for effective use of this metabolite in urine as a bioindicator of exposure to PAHs.

The pharmacokinetics of labeled and free pyrene have extensively been studied in rats (Withey *et al.*, 1991, 1992, 1993a,b, 1994). These data do not however yield comprehensive information on the rate of formation, amount in the body, and elimination kinetics of 1-OHP. Indeed, in these reports, the amount of metabolites was considered as the difference between total ^{14}C -pyrene and free pyrene determined by High Performance Liquid Chromatography (HPLC). On the other hand, in one of these published studies, it has been reported that 34 to 51% of an oral or intravenous dose of ^{14}C -pyrene was excreted in the urine over a period of six days following administration (Withey *et al.*, 1991). These findings are inconsistent with results obtained from the specific analysis of the apparent main metabolite of pyrene, 1-OHP (Boylard and Sims, 1964; Jacob *et al.*, 1982; Keimig *et al.*, 1983),

indicating that 1 to 3% of the administered pyrene was excreted as 1-OHP in rat urine over 24 or 48 hr postdosing and that elimination was almost complete in 24 hr (Jongeneelen *et al.*, 1985; Jacob *et al.*, 1989; Bouchard and Viau, 1996). The apparent discrepancy between these results emphasizes the need to examine, within a same experimental framework, the distribution and elimination of radiolabeled pyrene, in the parental form and as 1-OHP. This study was therefore undertaken to evaluate the kinetics of distribution and elimination of total radioactivity, and of pyrene and 1-OHP specifically, following intravenous injection of ^{14}C -pyrene in rats.

MATERIALS AND METHODS

Chemicals. Unlabeled pyrene (99% purity) and [4,5,9,10-¹⁴C]-pyrene were purchased from Sigma-Aldrich Chemical Co. (Oakville, ON, Canada). Specific activity was 61 mCi/mmol and purity was >95%. Reference standard of 1-OHP (>99% purity) was obtained from the NCI Chemical Carcinogen Reference Standards distributed by Midwest Research Institute (Kansas, MO). β -glucuronidase/arylsulfatase (100 000 Fishman U/ml and 800 000 Roy U/ml from *Helix pomatia*) was purchased from Boehringer Mannheim GmbH (Laval, Qc, Canada). Alkamuls EL-620 (formerly Emulphor 620) was kindly provided by Rhone-Poulenc (St-Hyacinthe, Qc, Canada). HPLC grade methanol, acetonitrile and dichloromethane were obtained from VWR-Canlab (Mississauga, ON, Canada). Ethyl acetate was obtained from Caledon Laboratories (Georgetown, ON, Canada). Naphthalene and ascorbic acid were purchased from Baker Analyzed Biochemical (J.T. Baker Chem. Co., Phillipsburg, NJ). Calcium oxide was purchased from Fisher Scientific (Ottawa, ON, Canada). BTS-450TM, Ready GelTM and Ready OrganicTM were obtained from Beckman Canada (Mississauga, ON, Canada).

Animals. Male Sprague-Dawley rats (Charles River Canada Inc., St-Constant, Qc, Canada) weighing 210 to 240 g were used. Lighting was maintained on a 12 hr light-dark cycle and room temperature was kept at $22 \pm 3^{\circ}\text{C}$. Prior to intravenous injection, rats were kept in plastic cages in groups of four and following the injection, animals were put in individual metabolic cages. The principles and guidelines of the Canadian Council on Animal Care were followed.

Rats were provided with food and water containing D-glucose (40 g/l), saccharin (1.5 g/l) and electrolytes (NaCl 0.9% and KCl 0.05%). Food was removed 16 hr prior to injection. Addition of sugar and electrolytes to the drinking water allowed to induce a polydipsic behavior with associated aqueous diuresis (Chouinard and Viau, 1992) permitting frequent urine collections and to maintain a caloric and electrolyte intake during fasting.

Animal treatment. Twenty four rats received a single intravenous dose of 50 $\mu\text{mol/kg}$ of [4,5,9,10- ^{14}C]-pyrene (20 $\mu\text{Ci/kg}$). Vehicle used for injection was a 20% Alkamuls: 80% isotonic glucose solution. Three ml of solution containing pyrene was injected per kg of body weight. Purity of ^{14}C -pyrene determined by separation on HPLC column, collection of fractions and counting was >99%. Specific activity was verified by liquid scintillation counting of aliquots of the stock solution.

Previous study on the urinary excretion kinetics of 1-OHP in the rat exposed to 0.5 to 50 $\mu\text{mol/kg}$ of pyrene has shown a strong dose-excretion relationship suggesting the absence of saturation of pyrene metabolism over the range of doses tested (Dodd and Viau, unpublished). The 50 $\mu\text{mol/kg}$ dose was chosen in the current report to ensure pyrene and 1-OHP levels would easily be detected in tissues. Furthermore, rats are known to exhibit similar 1-OHP urinary excretion kinetics as human volunteers (Viau *et al.*, 1995; Bouchard and Viau, 1996). This suggested that rats could be used as adequate models for the study of the toxicokinetics of 1-OHP.

Sampling. Groups of four rats were sacrificed by CO_2 inhalation at $t = 1, 2, 4, 8, 16$ and 24 hr postdosing. Blood (about 10 ml) was withdrawn by the abdominal artery and several tissues [liver,

kidney, lung, perirenal adipose tissue, muscle and GI tract (parenchyma and content of the stomach, small and large intestines and cecum)] were quickly removed, rinsed with saline, blotted dry, weighed and placed on ice. Urine and feces voided between the time of injection and sacrifice were collected. Thymol was added to the urine collection tubes prior to sampling. An additional group of four untreated rats were used as controls and blood was collected and tissues excised after a 24 hr-urine and feces collection period. All samples were kept at -20°C until analysis.

Analysis of ¹⁴C-pyrene equivalents, pyrene and 1-OHP.

Analysis of pyrene and 1-OHP. Urine samples were treated using the method of Jongeneelen *et al.* (1987). Blood, feces and tissues, except adipose tissue, were analyzed using a method adapted from Chipman *et al.* (1983) and Gerde *et al.* (1997). A 12.5% (w/v) homogenate of the whole tissue and a 6.25% (w/v) homogenate of all the feces voided by a given rat were prepared in a sodium acetate buffer (0.1M, pH 5.0). Aliquots of 4 ml of homogenate were transferred into Pyrex® tubes. On the other hand, samples of 500 µl of blood were buffered with 3.5 ml of sodium acetate buffer (0.1M, pH 5.0). Since 1-OHP conjugates with glucuronides and sulfates, blood, tissues and fecal homogenates were incubated overnight with 10 µl of β-glucuronidase/arylsulfatase in a shaking bath at 37°C. Samples were extracted twice with 4 ml of ethyl acetate saturated with water, shaken for 30 min and centrifuged for 20 min at 3000 rpm, 4°C. The organic extracts were combined and evaporated under a gentle nitrogen stream at 40°C. The residue was mixed vigorously with 70 mg of calcium oxide in 1.5 ml of saline to saponify and precipitate the lipids. The suspension was extracted twice with 4 ml of ethyl acetate, shaken for 30 min, centrifuged for 20 min at 3000 rpm, 4°C. The organic phases were combined, evaporated to dryness and the residue was redissolved in

1 ml of acetonitrile. For liver and GI tract analysis, samples were heated 5 min at 90°C prior to enzymatic hydrolysis and for these tissue analysis as well as for feces analysis, the lipid extraction step was not included.

For adipose tissue analysis, freshly homogenized tissues were hydrolyzed for 1 hr with 10 µl of β-glucuronidase/arylsulfatase, in a shaking bath at 37°C. If samples were hydrolyzed for a longer period of time, marked decrease of recovery of 1-OHP in the samples occurred unlike with the analysis of the other tissues. Samples were extracted twice with 4 ml of dichloromethane, shaken 20 min and centrifuged 20 min at 3000 rpm, 4°C. The organic phases were combined, evaporated under a gentle nitrogen stream at 35°C (i.e. below boiling point of dichloromethane) and redissolved in 1 ml of dimethylsulfoxide. Dilutions were prepared with acetonitrile.

Efficiency of the extraction of pyrene and hydrolyzed 1-OHP was determined using untreated rat blood, tissue, urine and feces samples spiked with authentic reference standards. Recovery of pyrene from spiked samples was 96 ± 4% in blood, 102 ± 6% in liver, 100 ± 9% in kidney, 104 ± 3% in lung, 108 ± 4% in muscle, 98 ± 6% in GI tract, 81 ± 4% in feces and 97 ± 9% in adipose tissue (mean ± SD for 6 samples i.e. triplicate of 2 levels of spiking). Recovery of 1-OHP from spiked samples was 87 ± 4% in blood, 89 ± 8% in liver, 70 ± 10% in kidney, 56 ± 8% in lung, 81 ± 7% in muscle, 73 ± 7% in GI tract, 93 ± 11% in feces and 78 ± 5% in adipose tissue (mean ± SE for 6 samples i.e. triplicate of 2 levels of spiking). Recovery of 1-OHP from spiked urine samples was 65 ± 7% (mean ± SD from 20 spiked samples). Results presented in this article were adjusted for recovery.

Analyses of pyrene and 1-OHP were performed using a HPLC system consisting of a model AS-100 automatic sampling system (Bio-Rad, Richmond, CA), a model 250 binary pump (Perkin-Elmer, Buckingham, England), and a Perkin-Elmer LS-40 fluorescence detector. The detector signal was recorded and treated with PE Nelson Turbochrom 3 software. For pyrene analysis, a 250 × 4.6 mm LC-PAH column (Supelco, Oakville, ON, Canada) was used. Analysis was performed in 12 min using a 90% acetonitrile: 10% water mobile phase and a flow rate of 1.5 ml/min. The column temperature was set at 32°C. Excitation and emission wavelengths were 333 and 390 nm, respectively. For 1-OHP analysis, a LC-18 Supelcosil 250 × 4.6 mm column (Supelco, Oakville, ON, Canada) was used. Urine analysis was performed in 25 min using a 71% methanol: 29% water mobile phase and a flow rate of 0.8 ml/min. Blood, tissue and feces analyses were conducted in 25 min using a 75% methanol: 25% water mobile and a flow rate of 1 ml/min. Column temperature was set at 32 °C. Excitation and emission wavelengths were 242 and 388 nm, respectively. Naphthalene (10 mg/l) was used as an external standard. Recovery and reproducibility of the chromatography was improved by the addition of 1 mg/l of ascorbic acid to the methanol eluent (Bouchard *et al.*, 1994). The injection volume was 20 µl throughout.

Analysis of ¹⁴C-pyrene equivalents. Blood samples (250 µl) were solubilized by adding 750 µl of BTS-450TM/isopropanol (1:2 v/v) and incubating for 1 hr at 40°C, and then decolorized by adding 500 µl of 30% hydrogen peroxide and incubating for 15 min at room temperature and 30 min at 40°C (Beckman Canada, Mississauga, ON, Canada). After neutralization with 70 µl of concentrated acetic acid, 10 ml of Ready GelTM were added to the samples. Tissue and feces homogenates (100 mg except muscle where 200 mg were used) were solubilized by adding 2 ml of BTS-450TM and

incubating for 5 hr at 40°C. Samples were neutralized with 70 μ l of concentrated acetic acid and 10 ml of Ready Organic™ or Ready Gel™ were added to tissues and feces samples, respectively. To aliquots of urine samples (1ml), 10 ml of Ready Gel™ were directly added prior to counting. After keeping the samples in the dark for at least 24 hr, levels of radioactivity were determined using a Tri-Carb Liquid Scintillation Analyzer Model 1500 (Packard Instrument Company, Downers Grove, IL).

Pharmacokinetic analysis. The time course of blood concentrations of pyrene was analyzed by a two-compartment model represented by the equation $C(t)=Ae^{-\alpha t} +Be^{-\beta t}$, where $C(t)$ is the concentration at time t , A and B are the preexponential coefficients and α and β are the hybrid rate coefficients for the initial and terminal phases, respectively. Calculation of the terminal elimination rate coefficient was based on the values of the last three points of individual curves and the initial rate coefficient was calculated using the method of residuals (Renwick, 1994). Half-lives were determined using the relationship $t_{1/2\alpha}=0.693/\alpha$ and $t_{1/2\beta}=0.693/\beta$. The intercompartmental exchange rate coefficients from compartment 1 to 2 and 2 to 1, k_{12} and k_{21} , the elimination rate constant for the central compartment, k_{elim} , and the apparent volume of distribution, V_d , were determined from the equations described by Renwick (1994).

Kinetics of ^{14}C -pyrene equivalents and 1-OHP in blood and tissues, and pyrene in tissues, were either monophasic or biphasic. Monophasic kinetics was represented by the equation $C(t)=C_0e^{-Kt}$, where C_0 is the concentration at time zero and K is the elimination rate constant, and elimination half-life was calculated using the equation: $t_{1/2}=0.693/K$. In the case of biphasic kinetics, $t_{1/2}$ was calculated for the terminal elimination phase (8 to 24 hr).

RESULTS

Kinetics of ¹⁴C-pyrene equivalents in blood and tissues

Time courses of ¹⁴C-pyrene equivalent concentrations in blood and in different tissues during the 24-hr period following intravenous administration of 50 μmol/kg of ¹⁴C-pyrene are shown in Fig. 1. Peak level was observed during the first sampling period for blood and tissues except the GI tract. Elimination curves for blood, liver, lung and muscle were parallel. Two apparent elimination phases were observed with an initial faster phase ranging between 1 and 8 hr and a terminal phase between 8 and 24 hr. Table 1 shows that the first order half-lives ($t_{1/2}$) calculated for the terminal elimination phase were similar for blood and these tissues.

Kinetics of ¹⁴C-pyrene equivalents in the GI tract and kidney were also biphasic with increasing or rather constant concentration values for up to 4 hr and progressively decreasing values thereafter. Elimination half-lives of ¹⁴C-pyrene equivalents in the GI tract and kidney, obtained for the 8- to 24-hr phase, were similar to those calculated in blood, liver, lung and muscle tissues (Table 1). On the other hand, disappearance of ¹⁴C-pyrene equivalents from adipose tissue was the most rapid. Elimination was monophasic from 1 to 24 hr with a first order $t_{1/2}$ value of 4.8 hr.

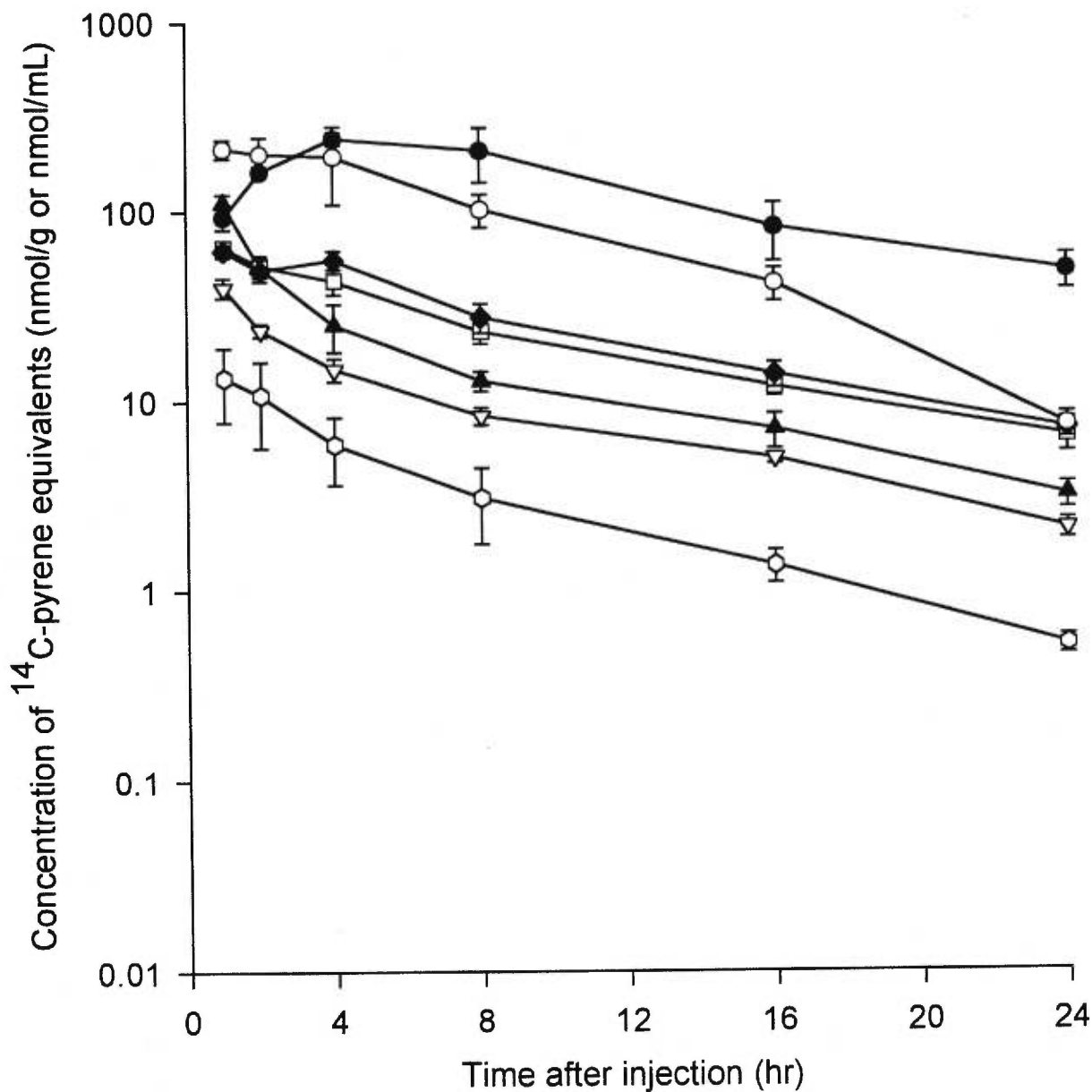


FIG. 1. Time course of blood and tissue concentrations of ¹⁴C-pyrene equivalents following intravenous administration of 50 $\mu\text{mol/kg}$ of ¹⁴C-pyrene in male Sprague-Dawley rats. Each *point* represents mean and *vertical bars* are standard deviations ($n = 4$). ● = gastrointestinal tract; ○ = adipose tissue; ◆ = kidney; □ = liver; ▲ = blood; ▽ = lung; ◇ = muscle.

TABLE 1

First order elimination half-lives of ^{14}C -pyrene equivalents, pyrene and 1-hydroxypyrene in blood and tissues following an intravenous injection of 50 $\mu\text{mol/kg}$ of ^{14}C -pyrene in male Sprague-Dawley rats.

Tissue	Mean first order elimination half-life (hr) (n=4)		
	^{14}C -pyrene ^a	pyrene ^a	1-OHP ^b
Gastrointestinal tract	7.5	3.1	----- ^c
Blood	8.0	4.0	6.7
Liver	8.7	3.8	6.2
Kidney	8.3	5.4	6.2
Lung	8.1	4.0	6.1
Muscle	6.2	3.6	5.2
Adipose tissue	4.8	4.9	----- ^d

^a Kinetics of ^{14}C -pyrene equivalents and pyrene in adipose tissue, and pyrene in the GI tract were monophasic and described by the equation $C(t)=C_0e^{-Kt}$, with $t_{1/2}=0.693/K$, where $C(t)$ and C_0 are the concentration at time t and zero, respectively, and K is the elimination rate constant. Kinetics of ^{14}C -pyrene equivalents and pyrene in blood and the other tissues were biphasic and summarized $t_{1/2}$ were calculated for the terminal elimination phase (8 to 24 hr).

^b Kinetics of 1-OHP was monophasic in liver and blood and described by the equation $C(t)=C_0e^{-Kt}$. Kinetics of 1-OHP was biphasic in the other tissues and $t_{1/2}$ were calculated for the terminal elimination phase (8 to 24 hr).

^c $T_{1/2}$ of 1-OHP in the GI tract was not calculated because 1-OHP concentrations increased until 8 hr postdosing and decreased very rapidly thereafter.

^d 1-OHP was barely detected in adipose tissue.

Kinetics of pyrene in blood and tissues

Profiles of pyrene concentrations over time in blood and tissues following intravenous injection of ^{14}C -pyrene (50 $\mu\text{mol/kg}$) are presented in Fig. 2. The time course of pyrene blood concentrations was described using a two-compartment model. Values of $t_{1/2}$ calculated for the initial (between 1 and 8 hr) and terminal (between 8 and 24 hr) elimination phases were 0.84 and 4.0 hr, respectively. Pharmacokinetic parameters were: mean preexponential coefficients, A and B, 40.50 and 1.50 $\mu\text{g/ml}$; hybrid rate coefficients, α and β , 0.01381 and 0.00292 min^{-1} ; apparent volume of distribution, V_d , 238.11 ml; intercompartmental exchange coefficients, k_{12} and k_{21} , 0.00124 and 0.00331 min^{-1} ; elimination coefficient, k_{elim} , 0.01219 min^{-1} . Intravenously-administered pyrene was rapidly distributed to the various tissues. Peak level in all tissues was observed during the first sampling period. Biphasic elimination kinetics of pyrene was also observed in all tissues except adipose tissue and GI tract. For the terminal elimination phase (between 8 and 24 hr), similar $t_{1/2}$ values were obtained for these tissues as shown in Table 1. In the GI tract, elimination of pyrene was monophasic and the first order half-life of 3.1 hr compared well with those obtained for the terminal elimination phase of pyrene in blood, liver, lung, kidney and muscle tissues. In adipose tissue, elimination of pyrene followed first order monophasic kinetics and a $t_{1/2}$ value of 4.9 hr was calculated. This time-course concentration profile of pyrene in adipose tissue was almost identical to the time course of ^{14}C -pyrene equivalent concentrations in this tissue. Elimination half-life of pyrene in adipose tissue also appeared similar to that of pyrene in the other tissues (Table 1).

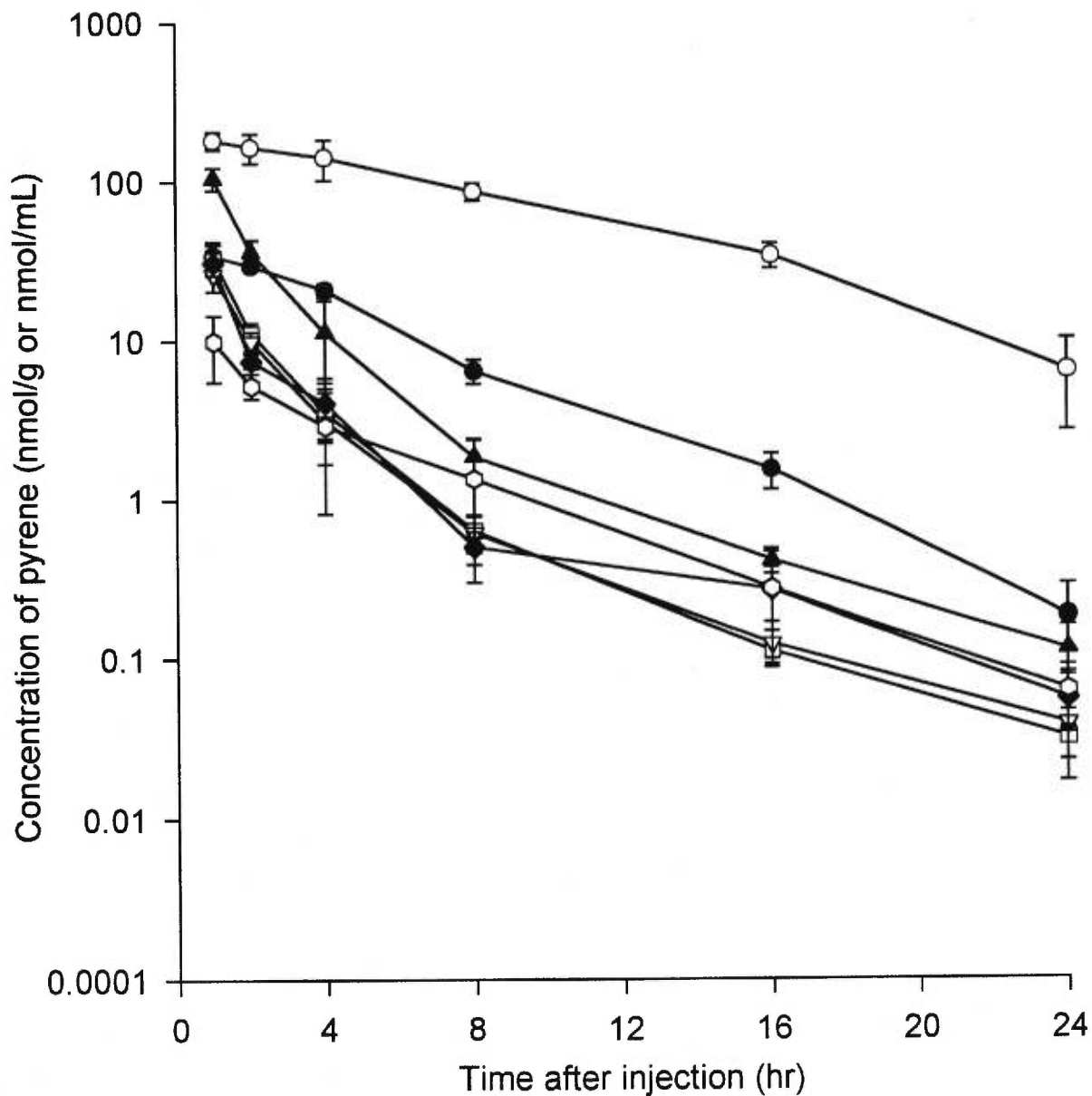


FIG. 2. Time course of blood and tissue concentrations of pyrene following intravenous administration of 50 $\mu\text{mol/kg}$ of ^{14}C -pyrene in male Sprague-Dawley rats. Each *point* represents mean and *vertical bars* are standard deviations ($n = 4$). ● = gastrointestinal tract; ○ = adipose tissue; ◆ = kidney; □ = liver; ▲ = blood; ▽ = lung; ◇ = muscle.

Kinetics of 1-hydroxypyrene in blood and tissues

The time course of 1-OHP blood and tissue concentrations following intravenous injection of ^{14}C -pyrene is depicted in Fig. 3. In blood and liver, peak concentration of 1-OHP occurred during the first and second sampling period, respectively. Elimination followed first order monophasic kinetics with respective mean $t_{1/2}$ values of 6.7 and 6.2 hr. Kinetics of 1-OHP in lung, kidney and muscle showed at least two phases and similar first order elimination half-lives of 1-OHP in these tissues were estimated for the 8- to 24-hr phase (Table 1). In the GI tract, 1-OHP concentrations increased progressively until 8 hr postdosing and decreased rapidly thereafter being barely measurable at 24 hr. An insufficient number of data points were available to calculate a reliable $t_{1/2}$ value for this latter elimination phase. On the other hand, 1-OHP was barely detected in adipose tissue at most sampling periods (≤ 3.9 pmol per g of tissue).

Urinary and fecal excretion of ^{14}C -pyrene equivalents, pyrene and 1-OHP

Total urinary and fecal excretion of ^{14}C -pyrene equivalents, pyrene and 1-OHP (all voids collected over 1, 2, 4, 8, 16 and 24 hr postdosing and expressed as a percentage of administered dose) is shown in Fig. 4. On average, 57.2 and 18.3% of the administered dose was recovered as ^{14}C -pyrene equivalents in the urine and feces, respectively, at 24 hr. Since excretion of unmetabolized pyrene in urine and feces was negligible, it appeared that pyrene was excreted virtually all in the metabolized form. However, molar fraction of the administered ^{14}C -pyrene excreted as 1-OHP in urine and feces was only 2.7 and 1.9%, respectively. Although 1-OHP in urine and feces represented only a small

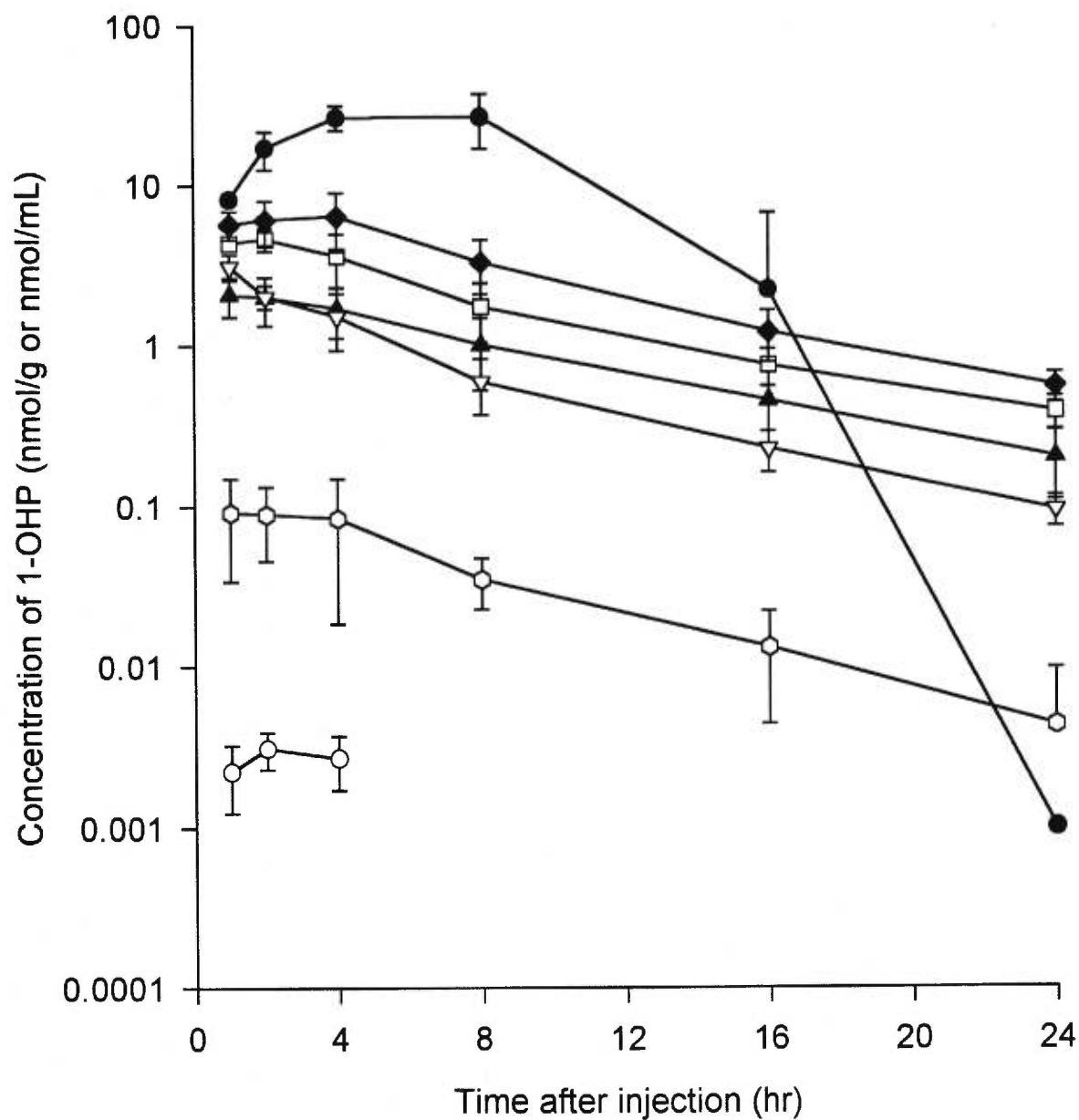


FIG. 3. Time course of blood and tissue concentrations of 1-hydroxypyrene following intravenous administration of 50 $\mu\text{mol/kg}$ of ^{14}C -pyrene in male Sprague-Dawley rats. Each *point* represents mean and *vertical bars* are standard deviations ($n = 4$). ● = gastrointestinal tract; ○ = adipose tissue; ◆ = kidney; □ = liver; ▲ = blood; ▽ = lung; ◇ = muscle.

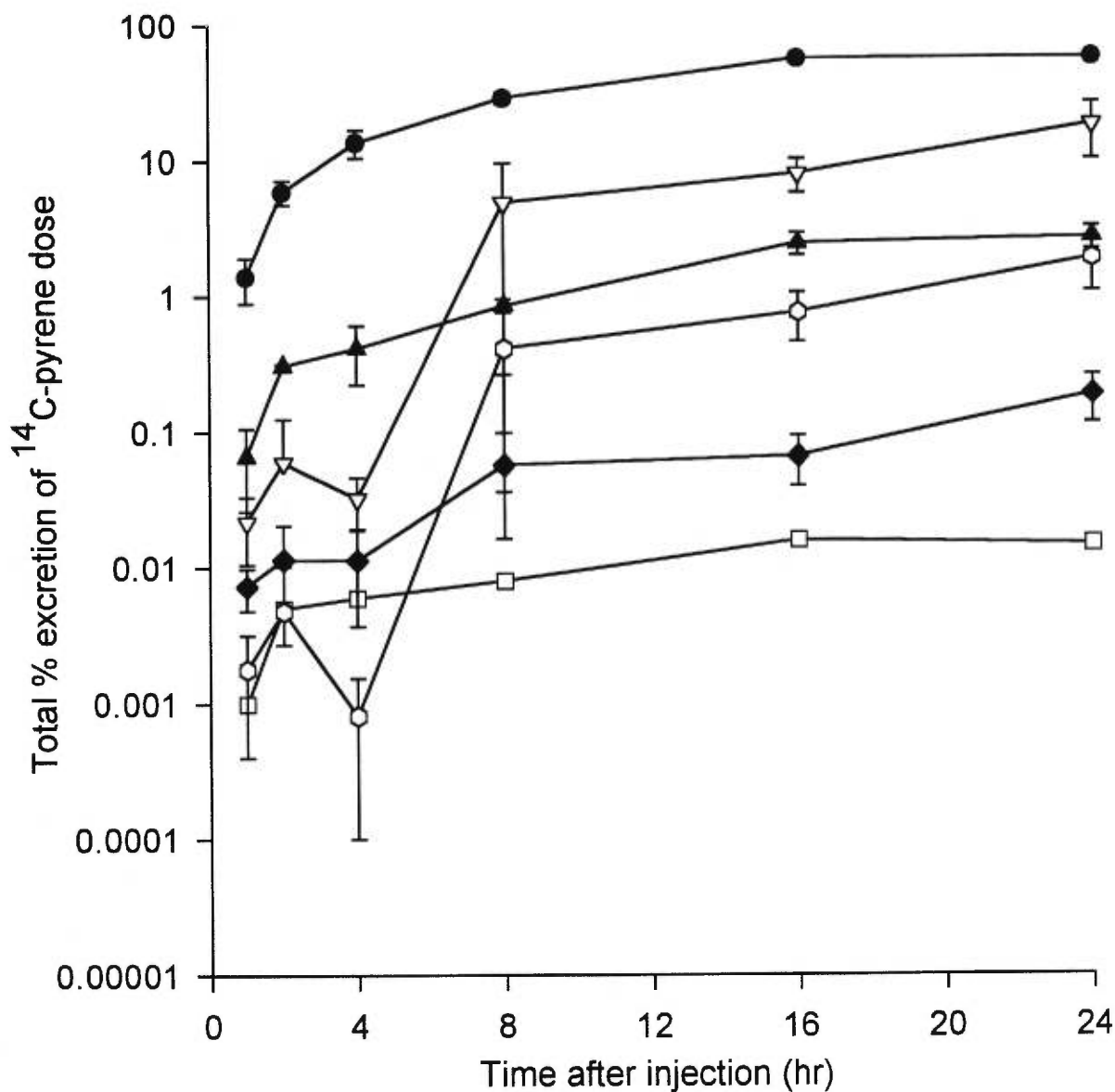


FIG. 4. Total urinary and fecal excretion of ^{14}C -pyrene equivalents, pyrene and 1-hydroxypyrene (1-OHP) (all voids collected over 1, 2, 4, 8, 16 and 24 hr postdosing and expressed as percentage of administered dose) following intravenous administration of $50 \mu\text{mol/kg}$ of ^{14}C -pyrene in male Sprague-Dawley rats. Each *point* represents mean and *vertical bars* are standard deviations ($n = 4$). ● = ^{14}C -pyrene equivalents in urine; ▽ = ^{14}C -pyrene equivalents in feces; ▲ = 1-OHP in urine; ◇ = 1-OHP in feces; ◆ = pyrene in feces; □ = pyrene in urine.

percentage of total ^{14}C -pyrene equivalents and hence of total metabolites excreted, the kinetics of 1-OHP cumulative excretion for both urine and feces resembled that of total ^{14}C -pyrene equivalents (Fig. 4).

Tissue distribution and urinary and fecal excretion of ^{14}C -pyrene equivalents, pyrene, 1-OHP and estimated total metabolites

Table 2 indicates the percentage of administered ^{14}C -pyrene recovered as ^{14}C -pyrene equivalents, pyrene and 1-OHP in blood, tissues and excreta over time. First, it is noteworthy that, at all time points, the sum of ^{14}C -pyrene equivalents makes up for the majority of the administered ^{14}C -pyrene. Second, it is clearly seen that ^{14}C -pyrene was rapidly distributed in adipose tissue. Indeed, at 1 hr, the adipose tissue exhibited the highest levels of ^{14}C -pyrene equivalents which represented essentially unmetabolized pyrene. Moreover, peak levels of ^{14}C -pyrene equivalents in adipose tissue was reached within 1 hr postdosing and declined rapidly thereafter with 96.4% of the accumulated pyrene in adipose tissue at 1 hr being eliminated at 24 hr postdosing.

At 1 hr, highest levels of ^{14}C -pyrene equivalents in non-adipose tissues were found in muscle and in blood, corresponding mostly to unmetabolized pyrene. More specifically, 75.1 and 94.7% of total ^{14}C -pyrene equivalents in muscle and blood, respectively, represented unmetabolized pyrene. At 1 hr, a significant percentage of dose was also recovered as ^{14}C -pyrene equivalents in the GI tract with 63% of total ^{14}C -pyrene equivalents corresponding to metabolized forms and 37% to unmetabolized

pyrene. With time, the fraction of metabolized pyrene in blood and these tissues rose progressively. This was also observed in the other non-adipose tissues (Table 3). In short, at 1 hr the largest part of the ^{14}C -pyrene dose was still in the unmetabolized form. However, at 24 hr, ^{14}C -pyrene dose was virtually all metabolized and excreted or present in the GI tract.

Elimination of ^{14}C -pyrene dose from the body was rapid. At 24 hr, 81.7% of the administered dose was recovered in the urine, feces and GI tract as ^{14}C -pyrene equivalents. However, only 4.6% of the dose was excreted as 1-OHP in urine and feces combined, at 24 hr. In other words, at that time point, the fraction of 1-OHP in urine to total metabolites in urine was 4.7% and the corresponding values in feces was 10.4%. Nonetheless, at all time points, 1-OHP in urine represented a constant fraction of total ^{14}C -pyrene equivalents and hence of total metabolites excreted in urine or in both urine and feces. In the urine, the average fraction of 1-OHP to total ^{14}C -pyrene equivalents for the various time points was $4.2 \pm 1.0\%$ (\pm SD). Correspondingly, the mean value of 1-OHP in urine to total ^{14}C -pyrene equivalents in both urine and feces was $3.8 \pm 1.0\%$ (\pm SD).

TABLE 2

Mean percentage of the administered dose recovered as ¹⁴C-pyrene equivalents, pyrene and 1-hydroxypyrene in blood, tissues and excreta at the various times of sacrifice (1, 2, 4, 8, 16 and 24 hr postdosing) following intravenous administration of 50 μmol/kg of ¹⁴C-pyrene in male Sprague-Dawley rats.

Tissue or biological fluid	Mean percentage of dose in tissues and biological fluids (n=4)														
	1		2		4		8		16		24				
	¹⁴ C	pyrene	¹⁴ C	pyrene	¹⁴ C	pyrene	¹⁴ C	pyrene	¹⁴ C	pyrene	¹⁴ C	pyrene			
Urine	1.390	0.001	0.066	5.910	0.005	0.313	28.763	0.008	0.854	55.827	0.016	2.461	57.217	0.015	2.710
GI tract	13.365	4.944	1.180	22.251	4.125	2.372	31.039	1.014	4.055	11.901	0.237	0.313	6.238	0.024	0.000
Feces	0.022	0.007	0.002	0.060	0.012	0.005	4.931	0.057	0.413	8.039	0.067	0.760	18.257	0.190	1.891
Blood	13.316	12.609	0.251	6.050	4.436	0.241	1.532	0.228	0.122	0.855	0.050	0.055	0.383	0.014	0.024
Liver	4.198	2.178	0.287	3.342	0.723	0.300	1.437	0.040	0.109	0.774	0.007	0.049	0.421	0.002	0.025
Kidney	0.972	0.493	0.089	0.775	0.120	0.096	0.436	0.008	0.053	0.211	0.004	0.019	0.111	0.001	0.009
Lung	0.410	0.276	0.032	0.228	0.097	0.020	0.075	0.006	0.005	0.046	0.001	0.002	0.020	0.000	0.001
Muscle	17.878	13.433	0.123	14.559	7.133	0.120	4.144	1.832	0.047	1.838	0.374	0.018	0.694	0.086	0.006
Adipose tissue	38.716	32.791	0.000	36.191	29.693	0.001	18.368	15.582	0.000	7.451	6.255	0.000	1.343	1.168	0.000
Total	90.268	66.732	2.030	89.366	46.344	3.467	90.724	18.774	5.657	86.943	7.011	3.676	84.684	1.499	4.665

TABLE 3

Estimated mean percentage of the administered dose recovered as total metabolites and fraction of total metabolites to ¹⁴C-pyrene equivalents in blood, tissues and excreta at the various times of sacrifice following intravenous administration of 50 µmol/kg of ¹⁴C-pyrene in male Sprague-Dawley rats.

Tissue or biological fluid	Estimated mean percentage of dose as metabolites and percentage of total metabolites to ¹⁴ C-pyrene equivalents in tissues and biological fluids (n=4)											
	Time after injection (hr)											
	1		2		4		8		16		24	
	Percentage of dose as metabolites	% of total metabolites to total ¹⁴ C	Percentage of dose as metabolites	% of total metabolites to total ¹⁴ C	Percentage of dose as metabolites	% of total metabolites to total ¹⁴ C	Percentage of dose as metabolites	% of total metabolites to total ¹⁴ C	Percentage of dose as metabolites	% of total metabolites to total ¹⁴ C	Percentage of dose as metabolites	% of total metabolites to total ¹⁴ C
Urine	1.389	99.93	5.905	99.92	13.654	99.96	28.755	99.97	55.811	99.97	57.202	99.97
GI tract	8.421	63.01	18.126	81.46	28.448	91.33	30.025	96.73	11.664	98.01	6.214	99.62
Feces	0.014	66.45	0.048	80.81	0.021	64.79	4.873	98.84	7.973	99.17	18.068	98.96
Blood	0.707	5.31	1.614	26.68	1.643	54.53	1.305	85.15	0.805	94.13	0.369	96.40
Liver	2.020	48.12	2.619	78.37	2.374	91.80	1.397	97.22	0.767	99.10	0.419	99.53
Kidney	0.479	49.30	0.655	84.51	0.790	92.55	0.428	98.14	0.207	98.01	0.110	99.19
Lung	0.134	32.66	0.131	57.50	0.098	78.27	0.069	92.63	0.044	97.58	0.019	97.95
Muscle	4.446	24.87	7.426	51.01	3.965	49.99	2.313	55.81	1.464	79.63	0.609	87.69
Adipose tissue	5.925	15.30	6.498	17.95	9.532	27.13	2.786	15.17	1.196	16.05	0.174	12.99
Total	23.536		43.022		60.525		71.950		79.931		83.184	

DISCUSSION

This report provides the first systematic study looking simultaneously at the tissue distribution and elimination kinetics of total radioactivity, and of pyrene and 1-OHP specifically, in rats exposed intravenously to ^{14}C -pyrene. It was clearly seen that ^{14}C -pyrene (50 $\mu\text{mol/kg}$) was rapidly distributed throughout the body, metabolized and readily excreted in the urine and feces. 1-OHP accounted only for a small fraction of total metabolites estimated from the difference between total radioactivity and pyrene levels. However, in general, kinetics of 1-OHP reflected that of total metabolites.

At 1 hr postdosing, highest levels of ^{14}C -pyrene equivalents were determined in adipose tissue which also corresponded to peak levels in that tissue reflective of the high lipophilicity of pyrene. Similar monophasic elimination kinetics of both ^{14}C -pyrene equivalents and pyrene in adipose tissue were depicted with identical $t_{1/2}$ values. These observations are in accordance with the fact that ^{14}C -pyrene equivalents in adipose tissue represent essentially unmetabolized pyrene. On the other hand, elimination kinetics of ^{14}C -pyrene equivalents in blood, liver, kidney, lung and muscle were biphasic with a terminal phase (between 8 and 24 hr) reflecting the elimination of metabolites. Indeed, this is suggested by the similar $t_{1/2}$ values for the terminal phase of ^{14}C -pyrene equivalents and 1-OHP elimination kinetics in non-adipose tissues which is consistent with the fact that at 8 hr, largest fraction of the dose has been metabolized (see Table 3).

In the GI tract, total ^{14}C concentration values increased between 1 and 4 hr and declined thereafter with an elimination half-life corresponding to that of total metabolites given that metabolized forms

were essentially present in the GI tract after 4 hr. Correspondingly, 1-OHP levels in the GI tract increased progressively until 8 hr postdosing and concentrations declined very rapidly thereafter although at a much faster rate than that of total metabolites which could suggest the presence of other metabolites in the GI tract such as thioether conjugates (Boyland and Sims, 1964) as determined in the case of benzo(a)pyrene (Weyand and Bevan, 1986).

Elimination of unchanged pyrene in blood, liver, kidney, lung and muscle occurred in a biphasic manner. The terminal elimination phase in these tissues was reflective of the elimination of unmetabolized pyrene in adipose tissue as suggested by the fact that $t_{1/2}$ value of pyrene in adipose tissue was similar to that of the terminal elimination phase of pyrene in blood and these non-adipose tissues (see Table 1). In other words, it appears that release of pyrene from adipose tissue was the rate-limiting step for the terminal half-life of pyrene in blood and non-adipose tissues. Furthermore, a monophasic elimination of pyrene from the GI tract was observed with a $t_{1/2}$ value corresponding to that of the other tissues analyzed and in particular adipose tissue thus indicating that elimination of pyrene from the GI tract also reflected that of pyrene from adipose tissue. It is noteworthy however that elimination of pyrene in adipose tissue was quite rapid. This suggests that pyrene does not accumulate in the adipose tissue of the rat as do polychlorinated biphenyls (Carrier, 1991).

Withey *et al.* (1992) observed that the largest fraction of pyrene body burden was found in adipose tissue at 6 hr postdosing. This is consistent with our results showing that at every time point, the largest fraction of total pyrene recovered in the body was determined in adipose tissue. Withey *et al.* (1992) also reported that the second highest level at 6 hr postdosing was observed in blood which

is in accordance with the data obtained in our study considering that in their study pyrene levels were not measured in muscle and GI tract. The pharmacokinetic parameters obtained in our study from the disappearance of pyrene from blood were generally close to those estimated by Withey *et al.* (1991) in Wistar rats. Mean values in our study and this other published study for a 44.5 $\mu\text{mol/kg}$ dose were α rate constant, 0.014 and 0.024 min^{-1} ; β rate constant, 0.0029 and 0.0040 min^{-1} ; V_d , 238 and 222 ml; k_{12} , 0.0012 and 0.0047 min^{-1} ; k_{21} , 0.0033 and 0.0055 min^{-1} ; k_{elim} , 0.012 and 0.018 min^{-1} .

With regard to 1-OHP, kinetics in blood and liver were monophasic while those in kidney, muscle and lung were biphasic. In the post-distributive phase, elimination curves of 1-OHP in blood and non adipose tissues were however parallel and $t_{1/2}$ values were similar (see Table 1). $T_{1/2}$ values were also similar to that obtained in a previous report for the terminal elimination phase of 1-OHP biphasic excretion kinetics in urine (Bouchard and Viau, 1996). This previous study demonstrated that phase I biotransformation was not the rate-limiting step in the excretion kinetics of 1-OHP in urine. Similarly, the current data showed that elimination rate of 1-OHP was slower than that of pyrene suggesting that the limiting factor in 1-OHP kinetics was not its rate of formation but rather its rate of elimination. Furthermore, it was observed that 1-OHP in blood and non-adipose tissues represented only a small percentage of the quantity of estimated total metabolites. However, kinetics of 1-OHP in blood and non-adipose tissues was, in general, similar to that of total metabolites (see Tables 2 and 3).

^{14}C -pyrene was rapidly excreted in both urine and feces following exposure and virtually all in the metabolized form with urinary excretion of total ^{14}C (57.2% of dose) being more important than fecal

excretion (18.2% of dose). Some radioactivity still remained in the GI tract at 24 hr (6.2% of dose). Although it has been documented that 1-OHP was the major metabolite of pyrene formed in vitro (Grover *et al.*, 1972; Jacob *et al.*, 1982) and detected in the urine of rats, pigs and rabbits (Boyland and Sims, 1964; Keimig *et al.*, 1983), it was determined in the current study that only 2.7 and 1.9% of dose were respectively excreted in urine and feces as 1-OHP. However, excretion kinetics of 1-OHP in urine and feces reflected that of total metabolites. It thus appears that 1-OHP remains a good indicator of the overall elimination of pyrene from the body.

It should be noted that the data presented in this report on ^{14}C -pyrene equivalent excretion in urine are consistent with the findings of Withey *et al.* (1991) who demonstrated that 29 to 40% of an intravenously administered ^{14}C -pyrene dose was recovered in urine as total ^{14}C over 24 hr postdosing. The current results concerning the specific analysis of 1-OHP in urine by HPLC are also in accordance with the findings of Jacob *et al.* (1989) and Jongeneelen *et al.* (1990) who reported that on average less than 2% of an acute dose was excreted as 1-OHP in urine. In a complementary experiment conducted in our laboratory, 24-hr urinary extracts from rats dosed with ^{14}C -pyrene were separated on a HPLC column, and fractions were collected and analyzed for radioactivity levels by liquid scintillation counting. It was observed that only a small proportion of total radioactivity found in urine had a retention time corresponding to that of 1-OHP. The bulk of the radioactivity corresponded to more polar metabolites and at least three other peaks were apparent.

Although no quantitative information were available, some authors have documented that other metabolites were present in the urine of rats namely N-acetyl-S-(4,5-dihydro-4-hydroxy-5-pyrenyl)-

L-cysteine, and sulfo- and glucurono-conjugates of 1,6- and 1,8-dihydroxypyrene and pyrene-4,5-dihydrodiol (Boyland and Sims, 1964). In the bile, in addition to the glucurono-conjugate of 1-OHP, N-acetyl-S-(4,5-dihydro-4-hydroxy-5-pyrenyl)-L-cysteine and the corresponding cysteine, cysteinylglycine and glutathione conjugates and glucurono-conjugates of pyrene-4,5-dihydrodiol have been detected (Boyland and Sims, 1964). Jacob *et al.* (1982) documented that 43% of the amount of pyrene metabolized in liver microsomes from rats corresponded to pyrene-4,5-dihydrodiol, an unidentified diphenol, 1,6-dihydroxypyrene and an unidentified triol.

On the other hand, data of the present study showed that as observed with estimated total metabolites, up to 8 hr postdosing, the largest fraction of total 1-OHP in the body and excreta was determined in the GI tract and at 16 and 24 hr postdosing, the major fraction of total 1-OHP was recovered in urine (67.0 and 58.1%, respectively) and feces (20.7 and 40.5%, respectively). Time course of estimated total metabolites or 1-OHP concentrations in the GI tract and total urinary and fecal excretion obtained in the present study suggests that a significant proportion of estimated total metabolites and 1-OHP initially present in the GI tract is reabsorbed. In a previous study, the urinary and biliary excretion of 1-OHP have been compared (Bouchard and Viau, submitted for publication). As obtained in the present study, it was determined that on average 2.7% of intravenously or orally administered pyrene was excreted as 1-OHP in urine over a 24-hr period postdosing. On average 8.9% of dose was recovered in bile over 24 hr. Since the current results indicate that only 1.9% of dose was excreted in the feces over 24 hr, it further appears that 1-OHP is subjected to enterohepatic recycling. Withey *et al.* (1991) also suggested that pyrene undergoes enterohepatic

recirculation and Weyand and Bevan (1986) provided evidence for enterohepatic recycling of benzo(a)pyrene metabolites.

Overall, ^{14}C -pyrene (50 $\mu\text{mol/kg}$) administered to rats was rapidly distributed throughout the body, metabolized and excreted in both urine and feces. At all time points, the largest fraction of total pyrene in the body and excreta was recovered in adipose tissue. The post distributive elimination phase of pyrene kinetics in blood and non-adipose tissues reflected the elimination of pyrene in adipose tissue. Furthermore, metabolite formation was very rapid and the rate of elimination of 1-OHP rather than its rate of formation appeared to be the limiting factor in its kinetics. Although 1-OHP in urine and feces represented only a small fraction of total amounts excreted and of estimated total metabolites, its excretion kinetics reflected that of total ^{14}C and thus of total metabolites which suggests that 1-OHP is a reliable indicator of exposure to and overall elimination of pyrene.

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CHAPITRE TROISIÈME

DISCUSSION GÉNÉRALE

Ce travail de recherche représente la première étude systématique sur la toxicocinétique du 1-hydroxypyrene (1-OHP). Il est utile de rappeler que sans connaissance adéquate de son comportement toxicocinétique, ce bioindicateur n'a qu'une valeur limitée pour évaluer la dose interne résultant de l'exposition aux hydrocarbures aromatiques polycycliques (HAP). Une revue de la littérature scientifique réalisée au début de ce projet avait montré que plusieurs chercheurs utilisaient déjà le 1-OHP dans l'urine comme mesure de la dose interne de pyrène et, par extension, des HAP totaux. Il était toutefois étonnant de constater la pauvreté des données sur la toxicocinétique de ce métabolite.

Jongeneelen et ses collaborateurs furent les premiers à proposer l'utilisation d'une mesure urinaire du 1-OHP pour évaluer l'exposition d'individus dans divers milieux de travail ou dans la population générale exposée aux HAP dans l'environnement. Cependant, aucune étude systématique sur la toxicocinétique de ce métabolite n'a été effectuée par ce groupe. Le groupe de Withey a étudié certains aspects de la toxicocinétique du pyrène chez des rats exposés de façon aiguë au composé marqué. La toxicocinétique du 1-OHP ne fut cependant pas incluse dans ces études. Au cours des dernières années, notre groupe de recherche a donc cherché à parfaire les connaissances sur le comportement cinétique de ce bioindicateur.

Il apparaissait en premier lieu que les données sur la cinétique d'excrétion urinaire du 1-OHP étaient incomplètes. Les premières études réalisées à cet effet par notre groupe de recherche ont porté sur la détermination de la cinétique d'excrétion urinaire détaillée du 1-OHP chez deux volontaires exposés par voies orale et cutanée au pyrène ou à la créosote (Viau *et al.*, 1995a; Viau et Vyskocil,

1995). À l'examen visuel, suite à la phase d'absorption, le profil cinétique du 1-OHP montrait au moins deux phases d'élimination. La demi-vie d'élimination apparente de premier ordre calculée pour la phase terminale d'élimination comprise entre 12 et 48 heures était comparable chez les deux volontaires et pour les deux voies d'exposition (soit environ 12 heures). Toutefois, une analyse plus détaillée des données a démontré qu'un modèle toxicocinétique classique à deux compartiments avec élimination de premier ordre ne permettait pas une simulation parfaite des résultats. La cinétique du 1-OHP semblait plutôt non linéaire de sorte qu'à mesure que les concentrations du 1-OHP diminuaient dans le compartiment central, c'est-à-dire le sang et les tissus en équilibre direct avec celui-ci, le taux d'élimination diminuait.

Suite aux études réalisées chez les volontaires, l'effet de la voie d'administration et de la dose sur la cinétique d'excrétion urinaire du 1-OHP a été étudiée plus en détail chez des rats exposés à des doses variant de 0,5 à 50 μmol de pyrène/kg par voies intraveineuse, orale et cutanée (Dodd et Viau, non publié). Des similitudes dans le comportement cinétique du 1-OHP dans l'urine de rats et d'humains ont pu être établies. À l'examen visuel, le profil d'élimination semblait biphasique comme il avait été observé chez l'humain. La phase terminale d'élimination (comprise entre 12 et 48 heures après exposition) était relativement parallèle aux différentes doses et après exposition par les différentes voies d'administration. La demi-vie apparente de premier ordre calculée pour la phase terminale d'élimination n'était que peu influencée par la voie d'administration (soit en moyenne 7,0, 6,3 et 8,6 heures après administrations intraveineuse, orale et cutanée, respectivement). Ceci résulte du fait que la vitesse d'absorption orale et cutanée du pyrène était plus rapide que la vitesse d'élimination du 1-OHP. Parmi les autres similitudes dans les données obtenues chez le rat et l'humain, il apparaissait

que la voie d'exposition influençait le pourcentage de la dose de pyrène excrétée dans l'urine sous forme de 1-OHP. En effet, la fraction de pyrène absorbée après administration cutanée était plus faible qu'après administration orale dû à l'effet de barrière protectrice de la peau. Toutefois, peu importe la voie d'administration, seul un faible pourcentage de la dose de pyrène était excrété sous forme de 1-OHP dans l'urine.

En parallèle avec les études sur la cinétique d'excrétion urinaire du 1-OHP, des expériences ont été effectuées sur l'effet de la voie d'administration et de la dose sur l'excrétion urinaire des métabolites du benzo(a)pyrène (BaP), en particulier le 3-hydroxyBaP (3-OHBaP) et le BaP-4,5-dihydrodiol (BaP-4,5-diol), chez des rats exposés à des doses de 2 à 60 μmol de BaP/kg par voies intraveineuse et orale (Article 1). Ceci visait à comparer le comportement cinétique du 1-OHP avec celui des métabolites du BaP. Rappelons que le BaP est le HAP le plus étudié et que son effet sur la santé s'avère davantage préoccupant que celui du pyrène. À l'heure actuelle, les métabolites du BaP sont toutefois difficilement mesurables dans l'urine humaine par les méthodes analytiques courantes mais le recours récent à des techniques très sensibles permettra éventuellement leur détection plus aisée. Les données de cette expérience ont montré que le profil temporel et les quantités excrétées des métabolites du BaP (3-OHBaP et BaP-4,5-diol) étaient similaires après administrations intraveineuse et orale suggérant que l'absorption orale du BaP était efficace et que les déterminants de la distribution, du métabolisme et de l'excrétion des métabolites du BaP étaient les mêmes pour ces deux voies. Par ailleurs, une bonne relation dose-excrétion était observée suggérant l'absence de saturation des systèmes enzymatiques impliqués dans la biotransformation du BaP, dans l'intervalle de doses utilisées dans l'expérience. Les mêmes observations ont pu être faites dans l'expérience sur la

cinétique d'excrétion urinaire du 1-OHP chez le rat (Dodd et Viau, non publié). Par contre, après application cutanée, l'excrétion urinaire absolue et relative des métabolites du BaP était différente de celle obtenue après administrations intraveineuse et orale. Ceci résulte entre autres du fait que le site majeur de biotransformation du BaP après administration cutanée serait la peau plutôt que le foie. En effet, l'absorption cutanée du BaP, en plus d'être conditionnée par un processus de diffusion passive, dépend en grande partie de la capacité de la peau à métaboliser ce composé. Évidemment, la peau agit également comme barrière protectrice limitant la pénétration du BaP. En comparant les résultats de cette expérience avec ceux de Dodd et Viau (non publié), il est par ailleurs intéressant de noter qu'il y a des différences apparentes dans le comportement cinétique du 1-OHP et des métabolites du BaP après application cutanée. Ceci s'explique par le fait que, contrairement au BaP, l'absorption du pyrène se fait principalement par diffusion passive et que seule une faible fraction est métabolisée au niveau de la peau. D'un autre côté, cette expérience a également permis de constater que la vitesse d'élimination variait d'un métabolite du BaP à l'autre. La vitesse d'élimination du BaP-4,5-diol était plus rapide que celle du 3-OHBaP peu importe la voie d'administration. Il apparaît donc qu'une mesure des ratios de concentrations des métabolites pourrait fournir des indications sur le moment et la voie majeure d'exposition.

De ces études mentionnées plus haut, nous avons très tôt réalisé que la biotransformation du pyrène en 1-OHP ou du BaP en ses métabolites ne semblait pas être un facteur critique dans la cinétique d'excrétion urinaire du 1-OHP ou des métabolites du BaP autant chez l'animal que chez l'humain. Ceci a pu être confirmé en déterminant le profil d'excrétion urinaire détaillé du 1-OHP et des métabolites du BaP après administration du composé parent (pyrène ou BaP) ou des métabolites eux-

mêmes chez le rat (Article 2). Cette expérience a par la même occasion permis de constater encore une fois des différences dans le profil cinétique de l'excrétion urinaire de certains métabolites du BaP suggérant qu'une mesure des ratios de concentrations de ces métabolites pourrait fournir de l'information sur le moment de l'exposition.

Les données rapportées précédemment ont également montré qu'un faible pourcentage de la dose de pyrène était excrété sous forme de 1-OHP dans l'urine. Il a alors semblé important de comparer l'excrétion urinaire et biliaire du 1-OHP suite à l'exposition à différentes doses de pyrène (de 1,5 à 100 $\mu\text{mol/kg}$) par voies intraveineuse et orale chez le rat (Article 3). Il a été observé que le pourcentage de la dose excrétée non seulement dans l'urine mais également dans la bile était faible. Ceci suggère que d'autres métabolites que le 1-OHP tel que mesuré dans l'étude sont présents en proportions significatives étant donné que le pyrène inchangé n'est retrouvé qu'à l'état de traces dans l'urine et les fèces. Les données de cette expérience sont en accord avec celles obtenues dans l'expérience sur la cinétique d'excrétion urinaire du 1-OHP chez le rat mentionnée précédemment (Dodd et Viau, non publié). La cinétique d'élimination urinaire et biliaire du 1-OHP après administration orale était semblable à celle observée après injection intraveineuse. Le pourcentage de la dose de pyrène excrétée dans l'urine ou la bile sous forme de 1-OHP après administration intraveineuse était également semblable à celui obtenu après administration orale et ce pourcentage était semblable aux différentes doses administrées. Une bonne relation dose-excrétion était donc observée suggérant l'absence de saturation dans l'absorption orale du pyrène ainsi que dans le métabolisme du pyrène et l'excrétion du 1-OHP aux doses utilisées dans l'expérience.

D'un autre côté, puisqu'en milieu de travail et dans l'environnement général, l'exposition se fait à des mélanges de HAP plutôt qu'aux HAP individuels, l'effet de l'exposition intraveineuse à des mélanges binaires et ternaires de pyrène, en présence de naphthalène ou de BaP sur la cinétique d'excrétion et sur la quantité excrétée du 1-OHP dans l'urine a aussi été étudié chez le rat (Article 4). La cinétique d'excrétion urinaire du 1-OHP n'était pas modifiée par la présence de naphthalène ou de BaP dans les mélanges. Par contre, des doses de 5 et 25 μmol de BaP/kg dans les mélanges causaient une augmentation significative dans la quantité de 1-OHP excrétée dans l'urine. Ceci est présumé dû à l'effet inducteur enzymatique du BaP. Il reste que la présence de naphthalène ou de la dose de 0,5 μmol de BaP/kg dans les mélanges n'influait pas la quantité de 1-OHP excrétée dans l'urine. Il apparaît donc que dans les conditions d'exposition environnementale ou en milieu de travail correspondant généralement à des doses équivalentes inférieures à la dose de 0,5 μmol de BaP/kg, l'exposition à des mélanges de HAP ne devrait pas altérer l'excrétion du 1-OHP. Il est intéressant de noter que les valeurs de demi-vie d'élimination du 1-OHP dans l'urine et de fraction molaire de la dose de pyrène excrétée dans l'urine sous forme de 1-OHP obtenues dans cette expérience sont semblables à celles d'expériences antérieures (Dodd et Viau, non publié; Article 2).

Malgré toutes ces études sur la cinétique d'excrétion urinaire du 1-OHP, il a également semblé important d'examiner le cheminement entre l'exposition au pyrène et l'élimination du 1-OHP. La cinétique de distribution et d'élimination du pyrène et du 1-OHP a donc été déterminée suite à l'administration intraveineuse de pyrène- ^{14}C chez le rat (Article 5). Cette expérience a montré que, suite à l'exposition au pyrène marqué, celui-ci était rapidement distribué dans les divers tissus. Une heure après exposition, la plus grande proportion de la dose se retrouvait au niveau des tissus

adipeux, essentiellement sous forme de pyrène inchangé. Son élimination des tissus adipeux était toutefois rapide suggérant l'absence d'accumulation dans ces tissus. Le pyrène était rapidement biotransformé (principalement au niveau du foie) et éliminé presque entièrement de l'organisme en 24 heures, sous forme métabolisée. La majeure partie des métabolites se retrouvait dans le contenu du tractus gastro-intestinal jusqu'à huit heures suivant l'exposition. Par après, bien qu'une certaine quantité du contenu gastro-intestinal était éliminée dans les fèces, une grande proportion était plutôt réabsorbée. Suite à cette recirculation entérohépatique, ces métabolites semblaient finalement être excrétés principalement dans l'urine. Par ailleurs, les résultats de cette expérience sont en accord avec ceux d'une expérience précédente suggérant que 7% du pyrène administré serait biotransformé et excrété sous forme de 1-OHP (Article 2). En effet, il a été observé que le 1-OHP (mesuré spécifiquement par chromatographie liquide à haute performance (HPLC)) représentait une faible proportion des métabolites totaux formés (estimés par la différence entre la mesure du ^{14}C total et celle du pyrène inchangé analysé spécifiquement par HPLC) et donc un faible pourcentage de l'excrétion totale. Il est toutefois important de souligner que la cinétique d'excrétion du 1-OHP dans l'urine et les fèces était semblable à celle des métabolites totaux reflétant donc l'élimination globale du pyrène de l'organisme.

Par ailleurs, il est à noter que dans quatre des cinq études expérimentales faisant l'objet de la thèse, les rats ont reçu de l'eau sucrée ou de l'eau sucrée et des électrolytes à boire pour induire une polyurie et permettre des collectes urinaires fréquentes. Il a été rapporté dans l'article 3 que de donner de l'eau ordinaire, de l'eau sucrée ou de l'eau sucrée et des électrolytes à des rats privés de nourriture pouvait influencer le pourcentage de la dose intraveineuse de pyrène excrétée sous forme

de 1-OHP dans l'urine. Cependant le profil cinétique urinaire (taux d'excrétion urinaire par unité de temps) demeurerait semblable peu importe que l'on donne de l'eau ordinaire, de l'eau sucrée ou de l'eau sucrée et des électrolytes, en collectant des urines à intervalles de six heures sur une période de 24 heures chez des rats pour lesquels la nourriture a été retirée six heures avant dosage. De plus, l'excrétion urinaire du 1-OHP dans une collecte de 24 heures a été comparée chez des rats recevant de l'eau ordinaire, de l'eau sucrée ou de l'eau sucrée et des électrolytes et privés de nourriture pendant les 6, 12, 24 ou 48 heures précédant une injection intraveineuse de pyrène. Le pourcentage de la dose de pyrène excrétée sous forme de 1-OHP dans l'urine était systématiquement quatre fois plus élevé chez des rats recevant de l'eau sucrée et des électrolytes que chez des rats buvant de l'eau ordinaire. Chez des rats buvant de l'eau sucrée, dépendant du moment où la nourriture était retirée avant l'injection, la quantité de 1-OHP excrétée dans l'urine se rapprochait soit de celle observée chez des rats recevant de l'eau sucrée et des électrolytes (retrait de la nourriture 6 et 48 heures avant dosage), soit de celle obtenue chez des rats buvant de l'eau ordinaire (retrait de la nourriture 24 heures avant dosage). Donc, chez des rats privés de nourriture, il apparaît a priori que de fournir de l'eau sucrée et des électrolytes à boire se rapproche davantage des conditions physiologiques que de donner de l'eau ordinaire seulement. Afin de confirmer ceci, il resterait important d'étudier la cinétique d'excrétion urinaire et les quantités excrétées de 1-OHP chez des rats recevant de la nourriture et de l'eau ordinaire ad libitum. Si on observait toutefois que les quantités de 1-OHP excrétées chez ces rats sont plus faibles que chez des rats privés de nourriture et recevant de l'eau sucrée ou de l'eau sucrée et des électrolytes, une explication plausible serait que la biodisponibilité des substances chimiques au niveau intestinal, et donc la réabsorption intestinale, devient plus importante chez des rats buvant de l'eau contenant du glucose, de la saccharine et des électrolytes.

En conclusion, ce projet de recherche apporte une contribution aux aspects plus fondamentaux de la toxicocinétique du 1-OHP. Il supporte en effet l'hypothèse que le 1-OHP dans l'urine est un bioindicateur utile pour l'évaluation de l'exposition à ce groupe de contaminants environnementaux préoccupants que sont les HAP. Évidemment, certaines études importantes restent à être effectuées afin de valider ce bioindicateur d'exposition. Entre autres, la modélisation pharmacocinétique à base physiologique aiderait à une meilleure compréhension des données expérimentales et pourrait être utilisée pour extrapoler les données expérimentales animales à l'humain. L'effet de l'exposition à des mélanges plus complexes sur l'excrétion quantitative du 1-OHP dans l'urine après exposition aiguë et en particulier après exposition répétée à des doses réalistes par rapport aux doses environnementales observables devrait également être examiné. Il s'agirait enfin de tenter de relier ce bioindicateur à l'apparition d'effets toxiques, dans une situation d'exposition répétée à des mélanges de HAP, afin d'établir si le 1-OHP, en plus d'être un indicateur fiable de l'exposition aux HAP, donne une bonne estimation du risque à la santé.

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