UNIVERSITÉ DU QUÉBEC À MONTRÉAL

CARACTÉRISATION DE LA VARIABILITÉ INTERINDIVIDUELLE DANS LA TOXICOCINÉTIQUE DES POLLUANTS ORGANIQUES PERSISTANTS CHEZ L'HUMAIN

THÈSE

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**Seules les abréviations anglaises ont été utilisées par souci d'uniformité.*

AA	Arachidonic acid
AUC	Area under the curve
β-HCH	β-Hexachlorocyclohexane
BMI	Body mass index
BRS	Behaviour Rating Scales
BSID-II	Bayley Scales of Infant Development (2nd edition)
CDC	Center for disease control and prevention
Cmax	Maximum concentration
DCFH-CA	2',7'-Dichlorodihydrofluorescein diacetate
DHA	Docosahexaenoic acid
HCB	Hexachlorobenzene
HOME	Home Observation for Measurement of the Environment
β-HCH	β-Hexachlorocyclohexane
Kow	octanol:water partition coefficient
LDH	Lactate dehydrogenase
LOD	Limit of detection
LTP	Lifetime toxicokinetic profile
MBDE	Mass balance differential equation
MNBC	Micronucleated binucleate cells
MTT	3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide
MDI	Mental Development Index
NDI	Nuclear division index
NPB	Nucleasmic bridges; Micronuclei
8-OHdG	8-hydroxy-2'-deoxyguanosine
OR	Odds ratio
PAF	Placental diffusion constant
PBDE	Polybrominated diphenyl ethers
PBDE-28	2,4,4'-Tribromodiphenyl ether

PBDE-47	2,2',4,4'-Tetrabromodiphenyl ether
PBDE-99	2,2'4,4',5-Pentabromodiphenyl ether
PBDE-100	2,2'4,4',6-Pentabromodiphenyl ether
PBDE-153	2,2'4,4',5,5'-Hexabromodiphenyl ether
PBDE-209	2,2',3,3',4,4',5,5',6,6'-Decabromodiphenyl ether
PBPK	Physiologically-based pharmacokinetic
PCB	Polychlorinated biphenyls
PCB118	2,3',4,4',5-pentachlorobiphenyl
PCB138	2,2',3,4,4',5'-hexachlorobiphenyl
PCB153	2,2',4,4',5,5'-hexachlorobiphenyl
PCB180	2,2',3,4,4',5,5'-heptachlorobiphenyl
POP	Persistent organic pollutants
p, p'-DDE	1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene
p, p'-DDT	1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane
TCDD	2,3,7,8-tetrachlorodibenzo-p-dioxin
TUNEL	TdT-mediated dUTP Nick-End Labeling
WPPSI-R	Wechsler Preschool and Primary Scale of Intelligence, Revised Edition

RÉSUMÉ

Les polluants organiques persistants (POP), dont les biphényles polychlorés (PCB) et le 1,1,1-trichloro-2,2-bis(*p*-chlorophényl)éthane (DDT), sont des contaminants lipophiles qui résistent à la dégradation et s'accumulent dans les tissus des organismes exposés. Malgré le fait que plusieurs de ces composés aient été retirés du marché, ils se retrouvent toujours en quantité mesurable dans les échantillons environnementaux et humains prélevés partout dans le monde. La toxicité de ces composés a été démontrée dans des études *in vitro* et chez les animaux. Parmi les différents effets de ces composés, on compte la perturbation endocrinienne, l'altération du neurodéveloppement, l'affaiblissement du système immunitaire ainsi que la promotion de certains cancers. Certains de ces effets ont aussi été observés dans des études épidémiologiques suite à des expositions accidentelles, occupationnelles et environnementales. Par contre, les études ne parviennent pas à un consensus quant à l'implication des POP dans l'étiologie de plusieurs problèmes de santé, notamment le cancer du sein et l'altération du neurodéveloppement suite à une exposition postnatale par l'allaitement.

Les études épidémiologiques sur les effets des POP se basent habituellement sur des échantillons de sang ou de lait maternel pour évaluer l'exposition. Dans le cas du cancer du sein, les échantillons sanguins sont généralement prélevés au moment du diagnostic ou quelques années avant. Quant à l'évaluation de l'exposition postnatale par l'allaitement, les études de neurodéveloppement ont jusqu'à maintenant estimé l'exposition des enfants en multipliant la concentration dans le lait maternel par la durée d'allaitement ou en prélevant des échantillons sanguins chez les enfants. Puisque les POP sont très persistants, ces échantillons sont considérés comme des marqueurs d'exposition chronique. Par contre, la capacité à représenter le profil d'exposition des sujets enrôlés dans des études épidémiologiques à partir de ces échantillons peut être gênée par différents phénomènes physiologiques pouvant changer les niveaux internes en POP, notamment les changements de poids corporel (ex : perte de poids, croissance) et l'allaitement. De plus, ces échantillons ne permettent pas d'évaluer l'exposition durant certaines périodes où les individus pourraient être plus susceptibles aux atteintes chimiques. Il était donc primordial de mettre sur pied une approche qui permette de détailler le profil d'exposition des individus afin d'évaluer les associations entre l'exposition durant certaines périodes critiques et la prévalence de certains problèmes de santé.

Les objectifs de ce projet étaient donc i) de développer des modèles pharmacocinétiques à base physiologique (PBPK) permettant d'estimer l'exposition à différentes périodes de la vie et ii) d'appliquer ces modèles au sein d'études épidémiologiques afin d'évaluer les associations entre l'exposition durant certaines fenêtres de temps et certains indicateurs de santé et de développement. La modélisation PBPK consiste en une représentation mathématique des phénomènes d'absorption, de distribution, de métabolisme et d'excrétion régissant le déplacement d'un composé chimique dans l'organisme exposé. Deux modèles PBPK ont été développés dans le cadre de ce projet : un modèle décrivant la cinétique des POP chez les femmes et un modèle décrivant le transfert postnatal mère-enfant par l'allaitement.

Le modèle PBPK de la femme permet de simuler la cinétique des POP en fonction des changements de poids, des grossesses et des allaitements. À partir d'une concentration sanguine au moment du diagnostic et d'informations récoltées dans les questionnaires épidémiologiques, le modèle PBPK génère des profils desquels il est possible d'extraire des concentrations à n'importe quel moment de la vie de la femme. Ce modèle a été utilisé afin de retracer des profils d'exposition aux PCB dans une étude cas-témoins sur le cancer du sein basée en France. Une analyse de corrélation a permis de quantifier la capacité d'une concentration sanguine au moment du diagnostic à représenter les concentrations simulées à différents moments de la vie. Cet exercice a permis de montrer que de tels échantillons sanguins perdent leur capacité à représenter l'exposition 1) plus la période d'intérêt est loin (ex : puberté) et 2) plus la durée d'allaitement totale est longue. Une limitation de ce modèle est que, puisque des données sanguines répétées n'étaient pas disponibles, sa validité n'a pu être établie.

Le modèle PBPK décrivant le transfert mère-enfant de POP a quant à lui été validé avec des données provenant d'une cohorte d'enfants inuits du nord du Québec. À l'aide du modèle PBPK, les profils d'exposition postnatale ont été tracés pour les enfants enrôlés dans l'étude. Leur concentration sanguine mesurée à environ six mois a ensuite été comparée aux valeurs simulées. Une analyse de corrélation de Spearman entre les niveaux simulés et mesurés a démontré que le modèle estimait adéquatement les niveaux de différents congénères de PCB ($r = 0.87 - 0.89$), de DDT/E ($r = 0.90/0.77$) et de HCB ($r = 0.83$). Suite à la validation du modèle, les profils générés pour le PCB-153 (le congénère de PCB prédominant dans les échantillons humain et fortement correlé aux autres congénères) ont été utilisés pour évaluer l'association entre les niveaux sanguins simulés à chacun des mois postnataux et des indicateurs d'attention et d'activité spontanée chez les enfants de 11 mois. L'exposition prénatale, telle qu'indiquée par les niveaux dans le sang du cordon ombilical, était associée à une réduction de l'attention alors que l'exposition postnatale, particulièrement durant le 4^{e} mois, était associée à une augmentation de l'activité spontanée.

Cette nouvelle approche a donc permis d'évaluer la validité des méthodes traditionnelles d'évaluation de l'exposition et d'identifier une nouvelle fenêtre de susceptibilité aux atteintes neurotoxiques des PCB. Les modèles PBPK présentés dans cette thèse sont déjà en application dans plusieurs autres études épidémiologiques afin de vérifier la reproductibilité des associations observées et de générer de nouveaux résultats.

Mots-clés : Modélisation pharmacocinétique à base physiologique (PBPK), polluants organiques persistants (POP), variabilité interindividuelle, exposition, cancer du sein, neurodéveloppement.

PROBLÉMATIQUE

Les polluants organiques persistants (POP) sont des contaminants ubiquitaires auxquels l'humain est exposé par différentes voies telles que la consommation d'aliments contaminés, le transfert intra-utérin ainsi que le transfert mère-enfant par l'allaitement. Parmi les POP les plus connus, on retrouve les biphényles polychlorés (PCB) et l'insecticide 1,1,1-trichloro-2,2-bis(*p*-chlorophényl)éthane (DDT), des molécules qui ont été retirées du marché au Canada et aux États-Unis dans les années '70 mais qui se retrouvent toujours dans les échantillons environnementaux et humains. Certains POP sont toujours produits aujourd'hui : certains sont synthétisés à des fins commerciales (ex : les polybromodiphényléthers [PBDE]) alors que d'autres sont des sous-produits d'activités industrielles comme la combustion de déchets (ex : les dioxines). Ces composés sont hautement lipophiles et faiblement métabolisés, entraînant ainsi leur bioconcentration dans l'environnement, leur bioamplification dans la chaîne alimentaire et leur bioaccumulation dans les organismes exposés. Plusieurs études *in vitro* et *in vivo* chez les rongeurs ont démontré qu'il existe une association entre l'exposition aux POP et l'avènement de divers problèmes de santé, notamment la promotion de certains cancers (ex : Norback et Weltman, 1985; Ptak *et al.*, 2010; Aube *et al.*, 2011), la perturbation du système endocrinien (ex : Kitamura *et al.*, 2005; Martin et Klaassen, 2010) et l'altération du neurodéveloppement (ex : Jolous-Jamshidi *et al.*, 2010; Fritsche *et al.*, 2005). Pourtant, les études épidémiologiques qui ont investigué ces associations chez l'humain n'ont pas toujours reproduit ces résultats de façon consistante, notamment dans le cas de plusieurs cancers et du neurodéveloppement suivant une exposition postnatale.

Jusqu'à présent, les études épidémiologiques portant sur les impacts d'une exposition aux POP sur la santé se sont basées sur des échantillons sanguins/tissulaires afin de déterminer les niveaux d'exposition interne. L'analyse de tels échantillons est une bonne approche pour estimer l'exposition interne aux POP sur une courte période de temps, mais la présupposition que les niveaux mesurés sont représentatifs du profil d'exposition complet ne fait pas consensus au sein de la communauté d'épidémiologistes. Par exemple, la majorité des études épidémiologiques ont utilisé les niveaux sanguins en POP au moment du diagnostic ou quelques années avant afin d'évaluer si ces composés ont un rôle dans l'étiologie du cancer du sein. Alors que les études

expérimentales ont clairement démontré la cancérogénicité de plusieurs POP, la majeure partie des études épidémiologiques sur le sujet n'ont pas rapporté d'association significative (Lopez-Cervantes *et al.*, 2004; Golden et Kimbrough, 2009; Negri *et al.*, 2003; Calle *et al.*, 2002). Plusieurs auteurs attribuent les résultats inconsistants aux erreurs dans l'évaluation de l'exposition (Brody et Rudel, 2003; Raaschou-Nielsen *et al.*, 2005; Lopez-Cervantes *et al.*, 2004). Dans ces articles, on rapporte qu'il existe probablement des fenêtres de susceptibilité aux atteintes chimiques, que ces périodes se situent probablement bien avant la détection du cancer (ex : enfance, puberté, grossesses), et que les niveaux en organochlorés mesurés au moment du diagnostic ne risquent pas de représenter l'exposition à ces moments. Ces inquiétudes sont bien fondées puisque plusieurs phénomènes tels que les changements de poids et l'allaitement influent sur les niveaux en POP et peuvent réduire la capacité d'un échantillon prélevé au moment du diagnostic à représenter l'exposition en bas âge (Imbeault *et al.*, 2002; Thomsen *et al.*, 2010).

Cette problématique se retrouve aussi au cœur des études sur les effets des expositions prénatales et postnatales aux POP sur le neurodéveloppement. L'exposition prénatale est habituellement définie par les niveaux retrouvés dans le sang de la mère durant la grossesse ou dans le cordon ombilical. Cette estimation de l'exposition est une approche raisonnable puisque la majorité des POP passent la barrière placentaire aisément et se distribuent de façon homogène dans les lipides de la mère et du fœtus, bien que certaines incertitudes subsistent quant aux effets de la prise de poids pendant la grossesse sur les niveaux de POP. La forte concordance entre les niveaux de POP mesurés dans les lipides du sang de la mère et du sang au cordon supporte la validité de cette approche (Jaraczewska *et al.*, 2006; Butler Walker *et al.*, 2003). Par contre, les méthodes employées jusqu'à présent pour estimer l'exposition postnatale par l'allaitement dans les études épidémiologiques ne rendent pas justice à la complexité de la cinétique des POP chez l'enfant. La majorité des études se sont basées sur des échantillons de lait maternel (ex : concentration dans le lait multipliée par la durée de l'allaitement) ou de sang prélevés chez les enfants. Ces estimations de l'exposition fournissent une approximation de l'exposition postnatale globale aux POP mais ne permettent pas une évaluation adéquate des niveaux internes durant différentes périodes du neurodéveloppement ayant lieu après la naissance. S'il existe des périodes spécifiques de vulnérabilité aux POP, il est fort probable que de telles estimations globales ne

permettent pas d'observer d'associations entre l'exposition et des déficits cognitifs, psychomoteurs ou comportementaux. Il serait donc important de connaître l'exposition durant différentes périodes du développement.

Effectuer un échantillonnage répété permettrait de documenter l'historique des niveaux sanguins. Par contre, cette approche est limitée par les coûts de prélèvement et d'analyse, ainsi que par des considérations éthiques liées aux prélèvements sanguins chez les enfants en bas âge. Une autre approche était donc nécessaire afin de mieux caractériser les profils d'exposition aux POP lors d'études épidémiologiques. C'est à cette fin que le présent projet de doctorat a été mis sur pied. Les objectifs principaux du projet étaient de développer des modèles pharmacocinétiques à base physiologique (PBPK) permettant d'estimer l'exposition à différentes périodes de la vie, de valider ces modèles lorsque des données répétées étaient disponibles et d'appliquer ces modèles au sein d'études épidémiologiques afin d'évaluer les associations entre l'exposition durant certaines fenêtres de temps et certains indicateurs de santé et de développement. Deux types de modèles PBPK ont été développés, soit un modèle d'exposition chez les femmes pour les études sur le cancer du sein et un modèle d'exposition postnatale aux POP pour les études sur le neurodéveloppement.

ÉTAT DES CONNAISSANCES

0.1. Polluants organiques persistants (POP)

Les polluants organiques persistants (POP) sont des molécules lipophiles résistantes à la dégradation et pouvant être bioamplifiées dans la chaîne alimentaire. En 2001, la convention de Stockholm a interdit la production de plusieurs POP (aldrine, chlordane, dieldrine, endrine, heptachlore, hexachlorobenzène, mirex, toxaphène, biphényles polychlorés) et restreint sévèrement l'utilisation du DDT, à l'exception des zones fortement touchées par la malaria comme l'Afrique du Sud. De plus, des recommandations ont été émises afin de limiter la production non-intentionnelle de dioxines et furanes. Malgré leur retrait du marché ou leur restriction, plusieurs études démontrent que les humains y sont toujours exposés et que ces contaminants peuvent causer des effets néfastes sur la santé. S'ajoutent à ces molécules des POP qui sont toujours sur le marché ou qui ont été retirés tout récemment tels que les polybromodiphényléthers (PBDE) auxquels les populations des États-Unis et du Canada sont particulièrement exposées. Quelques POP d'intérêt sont présentés brièvement afin de prendre connaissance de leur toxicité et de l'exposition humaine. Une emphase particulière est mise sur les biphényles polychlorés (PCB) puisqu'ils sont au cœur des projets présentés dans cette thèse.

0.1.1. Biphényles polychlorés (PCB)

Les PCB sont des molécules constituées d'un groupe biphényle auquel sont attachés de 1 à 10 atomes de chlore, formant ainsi une famille de 209 congénères caractérisés par le nombre et l'emplacement des atomes de chlore. Ces molécules ont connu plusieurs applications commerciales, notamment en tant qu'isolants pour les transformateurs électriques. Les PCB ont aussi été utilisés dans la production d'appareils électriques, de peinture et de pesticides. En Amérique du Nord, ces composés ont été mis sur le marché dans les années 1930 par la compagnie Monsanto, une production qui a connu son apogée aux Etats-Unis en 1970 avec 39 millions de kilogrammes produits annuellement. Suite à plusieurs rapports sur la persistance et la toxicité de ces substances, la fabrication et l'importation ont été interdits en Amérique du Nord à la fin des années 1970 (ATSDR, 2000).

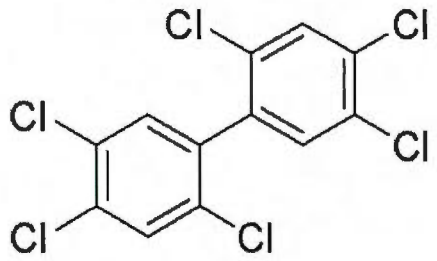

Figure 0.1. 2,2',4,4',5,5'-hexachlorobiphényle (PCB-153)

La toxicité des PCB a été documentée dans une quantité impressionnante d'études. Depuis 1970, plus de 12 000 articles scientifiques sur ces composés ont été publiés. Un vaste éventail d'effets des PCB ont été observés dans une multitude d'organismes. Des études *in vitro* sur différentes cultures de levures et lignées de cellules ont clairement démontré que certains congénères de PCB et leurs métabolites hydroxylés ont une activité endocrinienne, notamment en induisant ou en bloquant des réponses estrogéniques et/ou androgéniques (Svobodova *et al.*, 2009; Hamers *et al.*, 2011). Ces phénomènes ont été validés dans des études chez le rat où l'exposition aux PCB a été associée à une augmentation du poids de l'utérus chez les femelles ainsi qu'à une réduction du poids des testicules et une féminisation persistante du comportement chez le mâle (Hany *et al.*, 1999). En plus des hormones sexuelles, les PCB peuvent altérer l'homéostasie des hormones thyroïdiennes. Certains PCB hydroxylés ont démontré une affinité pour le récepteur (Freitas *et al.*, 2011; Kitamura *et al.*, 2005) et la protéine de transport des hormones thyroïdiennes, la transthyrétine (Lans *et al.*, 1993). On a d'ailleurs démontré que les ours polaires de l'île de Svalbard en Norvège sont contaminés par les PCB à un point tel que les sites de liaison à la transthyrétine sont complètement saturés (Gutleb *et al.*, 2010). Les niveaux en thyroxine (T4) dans le sérum des rats sont diminués significativement par une exposition aux PCB (Martin et Klaassen, 2010). Les PCB ont aussi la capacité de perturber le développement et le fonctionnement du système nerveux. La neurotoxicité des PCB a été démontrée dans plusieurs modèles *in vitro* dont les cellules progénitrices humaines (Fritsche *et al.*, 2005). L'exposition de ces cellules au PCB-118 induit la différentiation de ces dernières en oligodendrocytes, un phénomène normalement stimulé par l'exposition à l'hormone thyroïdienne triiodothyronine (T3) (voir figure 0.2). Des altérations du neurodéveloppement suite aux expositions prénatales et postnatales aux PCB ont aussi été mises en évidence dans des études chez le rat et le singe

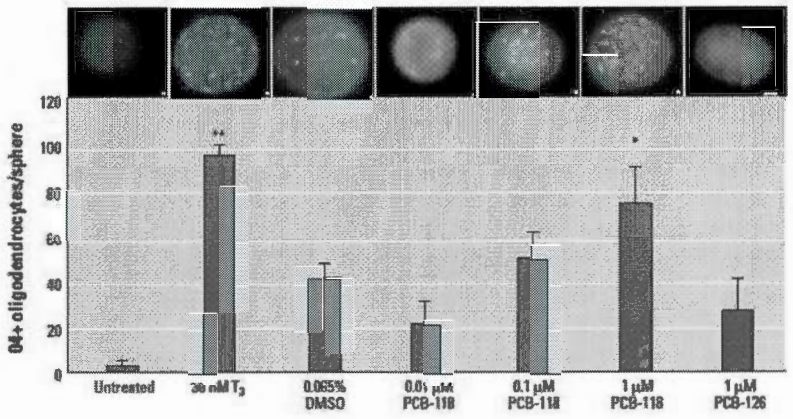

Figure 0.2. Effet de l'hormone thyroïdienne T3, du PCB-118 et du PCB-126 sur la différenciation des cellules progénitrices en oligodendrocytes (tiré de Fritsche *et al.*, 2005).

(Faroon *et al.*, 2001a). Certaines études *in vitro* on révélé que les PCB peuvent aussi altérer le système immunitaire en réduisant la phagocytose des lymphocytes humains (Mori *et al.*, 2006) et la prolifération des lymphocytes de souris et de mammifères marins (Levin *et al.*, 2005). Enfin, les PCB ont aussi identifiés en tant que cancérogènes. Des études sur des cellules du cancer du sein MCF-7 ont démontré que plusieurs congénères de PCB et leurs métabolites peuvent induire la prolifération cellulaire (Soto *et al.*, 1995; Ptak *et al.*, 2010). Une étude récente a révélé que les PCB peuvent aussi augmenter le pouvoir métastatique des cellules cancéreuses *in vitro* et *in vivo* chez la souris (Liu *et al.*, 2010).

Les PCB ont été intégrés dans plusieurs procédés industriels en raison de leur résistance aux variations de température. Ils ont entre autres été utilisés dans les échangeurs de chaleur pour la production d'huile de riz en Asie. En 1968, une grande quantité d'huile de riz a été contaminée par les PCB sur l'île de Kyushu au Japon. Suite à la consommation de l'huile contaminée, plus de 1800 personnes ont souffert de symptômes comme la chloracné, une pigmentation de la peau et des ongles, des sécrétions oculaires abondantes et une insensibilité dans les membres (Ikeda, 1996). Ce syndrome a été nommé « Yusho », ou maladie de l'huile en japonais. Une contamination d'huile de riz a également eu lieu à Taichung à Taïwan en 1979. Un syndrôme semblable a été observé, et nommé « Yu-Cheng » (même signification que Yusho). Les victimes de Yusho et Yu-cheng ont été suivies depuis les incidents afin de documenter les effets à long terme résultant d'une exposition aigüe aux PCB. Une réduction du quotient intellectuel et

certains problèmes comportementaux ont été observés chez les enfants des mères exposées à Taïwan (Chen et al. 1994). Un excès dans la mortalité due au cancer du foie a aussi été rapporté pour l'accident Yusho, un phénomène qui a aussi été observé dans les études occupationnelles chez des Américains ayant travaillé dans les usines de transformateurs électriques alors que les PCB étaient employés dans leur fabrication (Faroon *et al.*, 2001b). Les victimes de Yu-Cheng et Yusho ont vu leur système immunitaire affaibli à la suite de l'intoxication, ce qui a augmenté la prévalence des infections chroniques aux bronches chez les victimes de Yusho (Aoki, 2001). Des baisses dans les niveaux des hormones thyroïdiennes T3 et T4 ont aussi été enregistrées chez les victimes de Yusho. Malgré le fait que des dioxines et furanes aient aussi été détectées dans les échantillons d'huile contaminée, le patron d'effets causés par ces intoxications massives est très similaire à celui rapporté pour les expositions aux PCB *in vitro* et *in vivo* chez l'animal, ce qui supporte l'implication des PCB dans l'étiologie de ces effets.

Alors que des expositions aigües survenues lors d'accidents ou en milieu de travail ont touché certains groupes de personnes, les expositions environnementales aux PCB concernent quant à elles la majorité des humains. La présence de ces composés dans la majorité des échantillons de sang, de tissu ou de lait maternel prélevés à l'échelle mondiale confirme cette contamination

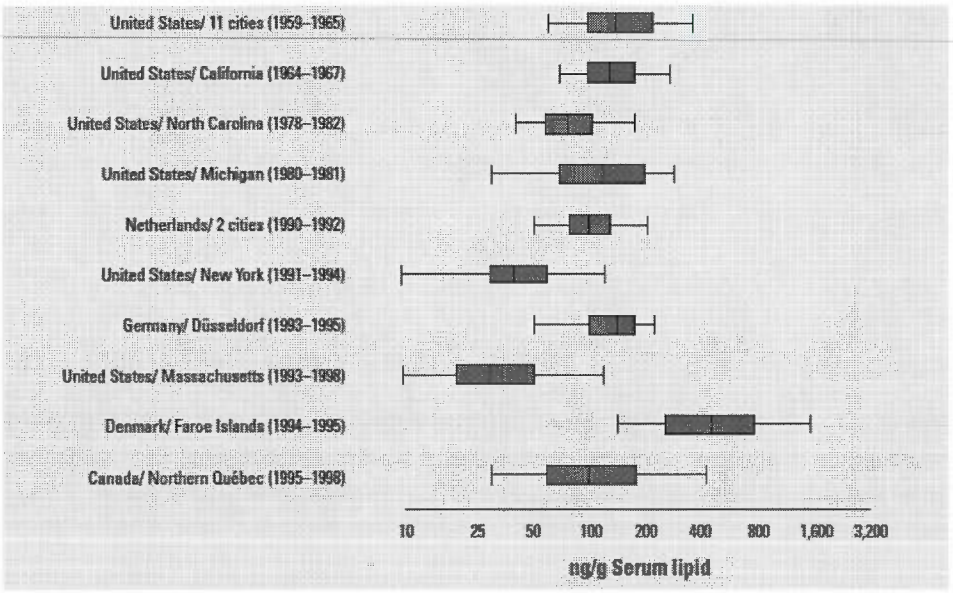

Figure 0.3. Niveaux sériques en PCB-153 dans dix études sur le neurodéveloppement chez l'humain (tiré de Longnecker *et al.*, 2003).

globale. Longnecker *et al.* (2003) ont comparé les niveaux en PCB-153 (congénère le plus prévalent dans les tissus humains) mesurés dans les échantillons prélevés dans le cadre de dix études sur le développement des enfants. La figure 0.3 démontre clairement un déclin dans les niveaux entre les années '60 et '90 aux États-Unis. On peut aussi remarquer des niveaux plus élevés en Europe et dans le nord du Québec qu'aux États-Unis durant les années '90. Les niveaux importants mesurés dans les populations des Îles Faroe et du nord du Québec sont le résultat i) du transfert des PCB du sud vers le nord par le mouvement des masses d'air chaud vers les pôles (effet sauterelle) (Oehme, 1991) et ii) d'une alimentation traditionnelle incluant du poisson et des mammifères marins se retrouvant au sommet de la chaîne alimentaire et contenant conséquemment de grandes quantité de PCB (Muir *et al.*, 1988).

L'exposition environnementale aux PCB a suscité l'intérêt de plusieurs groupes d'épidémiologistes. Plusieurs études épidémiologiques ont rapporté une association entre les concentrations de PCB dans le sérum et les niveaux d'hormones thyroïdiennes. Chez l'adulte, une association négative a été observée entre les niveaux sériques en PCB et en T3 et T4 totaux, bien que cette association n'ait pas été rapportée par toutes les études sur le sujet (Salay et Garabrant, 2009). Certains résultats suggèrent que la perturbation des hormones thyroïdiennes diffère selon le sexe (Salay et Garabrant, 2009). Puisque les hormones thyroïdiennes maternelles durant la grossesse sont cruciales pour le développement du cerveau de l'enfant, l'effet des PCB sur les niveaux de T3, de T4 et de thyrotrophine (TSH) a été investigué à plusieurs reprises. Les niveaux sanguins en PCB durant la grossesse ont été associés à une réduction dans les niveaux de T3 totaux (Takser *et al.*, 2005; Alvarez-Pedrerol *et al.*, 2009) et de T3 libre (Chevrier *et al.*, 2008), et une augmentation dans les niveaux de T4 libres (Alvarez-Pedrerol *et al.*, 2009). Les concentrations sériques de PCB chez les femmes enceintes ont aussi été associées à une modulation des niveaux d'hormones thyroïdiennes chez les nouveaux nés. Chevrier *et al.* (2007) ont observé une relation entre la somme des PCB pouvant induire les enzymes glucuronosyltransférases (enzymes métabolisant les hormones thyroïdiennes) et une augmentation de la TSH. Une baisse dans les niveaux en T4 totaux a été associée aux niveaux en PCB-153 dans le sang au cordon (Herbstman *et al.*, 2008). La perturbation des hormones thyroïdiennes rapportée dans les études chez les animaux peut donc être observée dans les populations exposées à des niveaux environnementaux de PCB.

Plusieurs cohortes de naissance ont été mises sur pied afin d'évaluer l'effet des expositions pré- et postnatales aux PCB sur le neurodéveloppement des enfants. Maintes approches ont été employées afin de caractériser l'exposition prénatale. Les études ont utilisé les niveaux en PCB dans le sang des femmes enceintes, dans le sang au cordon ombilical, dans le placenta, dans le lait maternel peu après l'accouchement ou la fréquence de consommation de poisson pour quantifier l'exposition intra-utérine. Ces estimations de l'exposition prénatale ont été associées à plusieurs indicateurs de développement cognitif, psychomoteur et comportemental. Un test administré dans les jours suivant la naissance, le Brazelton's Neonatal Behavioral Assessment Scales (NBAS), a permis de détecter des effets d'une exposition prénatale aux PCB sur plusieurs domaines du comportement des enfants (Stewart *et al.*, 2000; Sagiv *et al.*, 2008; Suzuki *et al.*, 2010a). L'exposition prénatale aux PCB a aussi été associée à une réduction des scores sur un test de mémoire visuelle, le Fagan Test of Infant Intelligence administré durant la première année de vie (Jacobson *et al.*, 1985;Darvill *et al.*, 2000), ainsi que sur les échelles de développement congnitif et psychomoteur du Bailey Scales of Infant Development habituellement administré entre six mois et trois ans (Korrick et Sagiv, 2008; Schantz *et al.*, 2003; Ribas-Fito *et al.*, 2001). Quelques études rapportent que les effets d'une exposition prénatale aux PCB sur le neurodéveloppement de l'enfant peuvent persister au-delà de l'enfance.

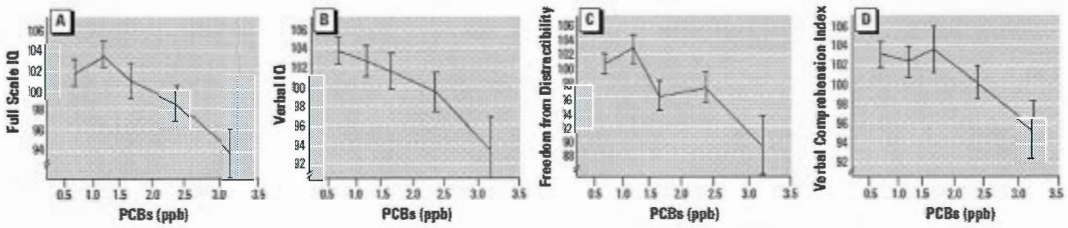

Figure 0.4. Association entre les niveaux en PCB dans le tissu placentaire et des scores de quotient intellectuel global (A), de quotient intellectuel verbal (B), de distractibilité (C) et de compréhension verbale (D) chez des enfants de 9 ans (tiré de Stewart *et al.*, 2008)

Entre autres, les PCB prénataux ont été associés à une réduction des fonctions intellectuelles des enfants. La figure 0.4 démontre clairement l'effet d'une exposition prénatale sur les capacités intellectuelles et d'attention à l'âge de 9 ans, un phénomène pouvant avoir des répercussions importantes sur le rendement scolaire des enfants (Stewart *et al.*, 2008). Les résultats de la cohorte du Michigan indiquent que l'exposition aux PCB peut réduire significativement la

mémoire et l'attention des enfants de 11 ans (Jacobson et Jacobson, 1996). Dans une étude sur des enfants nés près d'un site contaminé aux PCB à New Bedford aux États-Unis, l'exposition prénatale a été associée à des comportements semblables à ceux observés chez les enfants atteints du trouble déficit de l'attention hyperactivité (Sagiv *et al.*, 2010). Ces altérations dans le développement du cerveau des enfants exposés prénatalement à des niveaux environnementaux de PCB confirment la neurotoxicité observée dans les études *in vitro* et chez les modèles animaux.

Les études sur l'exposition postnatale par l'allaitement sont équivoques quant à la relation entre l'exposition aux PCB et le neurodéveloppement (Jorissen, 2007). Alors que deux études européennes ont observé une relation entre l'exposition postnatale aux PCB et une réduction des scores sur les échelles du Bailey Scales of Infant Development (Koopman-Esseboom *et al.*, 1996) et du Kaufman Assessment Battery for Children (Walkowiak *et al.*, 2001), aucune association de la sorte n'a été rapportée dans les études américaines (Gladen *et al.*, 1988; Jacobson et Jacobson, 1996; Jacobson *et al.*, 1985; Pan *et al.*, 2009). Cette inconsistance est peut-être liée aux limitations des approches d'évaluation de l'exposition postnatale employées jusqu'à présent, soit en multipliant la concentration de PCB dans le lait maternel par la durée d'allaitement, soit en mesurant les niveaux de PCB dans le sang de l'enfant. Les estimations obtenues à partir de ces approches ne rendent pas compte de l'exposition à différentes périodes du développement postnatal, ce qui peut grandement limiter la capacité à détecter des associations entre l'exposition et les indicateurs de développement cognitif, moteur ou comportemental.

Le cancer du sein est un autre domaine de recherche où les effets observés chez les animaux ne sont reproduits chez l'humain que dans certaines études. En effet, la majorité des études épidémiologiques n'ont pas détecté d'association significative entre l'exposition aux PCB et la prévalence du cancer du sein (études revues par Golden et Kimbrough, 2009; Negri *et al.*, 2003; Moysich *et al.*, 2002; Calle *et al.*, 2002). Les études cas-témoins sur le cancer du sein on basé leurs analyses sur des niveaux de PCB dans des échantillons prélevés chez les sujets au moment du diagnostic du cancer ou quelques années auparavant. Cette approche pour évaluer l'exposition des femmes aux PCB a été pointée du doigt par plusieurs épidémiologistes comme

étant probablement responsable de cette incapacité à déceler une association entre l'exposition et l'incidence du cancer du sein. Dans la revue de littérature publiée par Salehi *et al.* (2008), on rapporte que les méthodes utilisées jusqu'à présent ne prennent pas en compte la période de latence entre l'exposition et le développement du cancer, et que la difficulté d'obtenir des mesures précises des niveaux durant des périodes où le tissu mammaire pourrait être plus susceptible aux atteintes chimiques est probablement en cause dans l'inconsistance dans les résultats des études. Raaschou-Nielsen *et al.* (2005) ont tenu des propos semblables en donnant comme exemple que si les organochlorés comme les PCB exercent leur effet cancérogène durant une fenêtre de susceptibilité telle que la puberté, il est incertain qu'une concentration mesurée au moment du diagnostic puisse représenter l'exposition durant cette période.

0.1.2. Pesticides organochlorés

Les pesticides organochlorés représentent une famille de composés utilisés pour le contrôle des parasites. La majorité des POP sur lesquels la convention de Stockholm a statué font partie de ce groupe de substances produites afin de combattre les insectes (mirex, lindane, endrine, heptachlore, aldrine, dieldrine, chlordane, toxaphène, DDT) et les champignons (hexachlorobenzène). Alors que l'usage de ces pesticides est restreint depuis plusieurs années, l'Organisation Mondiale de la Santé a émis en 2006 un rapport dans lequel on recommande la pulvérisation résiduelle intérieure de DDT pour la lutte vectorielle contre la malaria dans les régions fortement touchées par la maladie, notamment en Afrique du Sud. Des effets sur la santé ont été rapportés pour plusieurs de ces molécules.

Une étude de Li *et al.* (2008a) sur des levures exprimant certains récepteurs hormonaux humains a révélé que plusieurs organochlorés peuvent perturber la signalisation endocrinienne en agissant en tant qu'agonistes ou antagonistes des récepteurs à œstrogènes, à progestérone ou à androgènes. Les résultats de cette étude ont démontré que le 1,1-dichloro-2,2-bis(*p*-chlorophényl)éthylène (DDE), principal métabolite du DDT, peut agir en tant qu'agoniste du récepteur à oestrogènes ERα et en tant qu'antagoniste des récepteurs à androgènes et à progestérone. Le DDT peut quant à lui avoir une action à la fois agoniste sur le récepteur ERα et antagoniste sur le récepteur à progestérone. L'hexachlorobenzène ne présente aucune activité

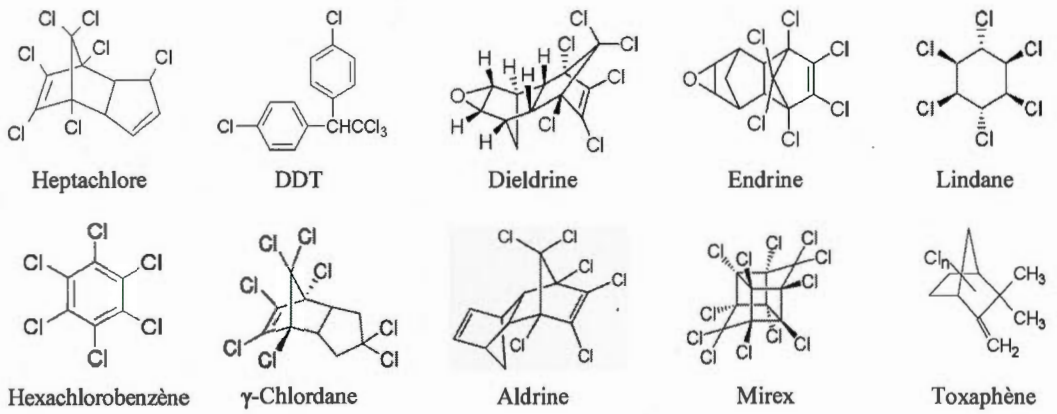

Figure 0.5. Pesticides organochlorés

agoniste sur ces récepteurs, mais il est antagoniste des récepteurs à androgènes et à oestrogènes ERRγ. Plusieurs études *in vivo* chez le rat ont démontré qu'une exposition à différents organochlorés peut moduler les niveaux en hormones thyroïdiennes (études revues par Schantz et Widholm, 2001). En plus de perturber le système endocrinien, les organochlorés peuvent affecter le système immunitaire. L'exposition *in vitro* de cellules immunitaires humaines au DDT réduit significativement l'activité lytique des lymphocytes NK (Udoji *et al.*, 2010) et altère les fonctions des macrophages et du système du complément (Dutta *et al.*, 2008). Des effets cancérogènes ont également été observés avec certains organochlorés. La prolifération des cellules MCF-7 du cancer du sein peut être induite par l'exposition à plusieurs composés comme le DDT et ses métabolites, la dieldrine et le toxaphène (Soto *et al.*, 1995).

L'exposition aux pesticides organochlorés chez l'humain a fait l'objet de plusieurs études épidémiologiques. Ces composés ont été associés à une panoplie d'effets sur les systèmes endocrinien, immunitaire et neuronal. Les niveaux en DDE durant la grossesse ont été positivement associés aux concentrations de TSH et négativement aux niveaux de T3 libre (Lopez-Espinosa *et al.*, 2009). Une réduction des niveaux en T3 totaux et une hausse dans la T4 libre ont été observées en relation avec les concentrations sanguines en hexachlorobenzène durant la grossesse. Une étude a démontré que les niveaux en β-HCH dans le sang au cordon sont associés avec une augmentation dans la concentration en TSH dans le sérum des nouveaux nés (Alvarez-Pedrerol *et al.*, 2008a) un phénomène ayant été associé à une réduction des

aptitudes cognitives et une augmentation des risques de développer des comportements d'inattention et d'hyperactivité chez les enfants de quatre ans (Alvarez-Pedrerol *et al.*, 2007; Freire *et al.*, 2010). Une association inverse a aussi été observée entre les concentrations de DDT et de β-HCH mesurées dans le sang d'enfants de quatre ans et les niveaux de T3 (Alvarez-Pedrerol *et al.*, 2008b).

Le système immunitaire est aussi la cible des atteintes chimiques par les organochlorés chez l'humain. Les résultats d'une étude récente publiée par Sunyer *et al.* (2010) ont démontré que les niveaux sanguins de DDE durant la grossesse sont positivement associés aux infections du système respiratoire chez les enfants de six à 12 mois. L'exposition prénatale au DDE a aussi été mise en relation avec une réduction des niveaux d'éosinophiles sériques (Glynn et al. 2008). Une augmentation dans le nombre d'infections durant la première année, les otites en particulier, a été observée en relation avec les niveaux prénataux de DDE et d'hexachlorobenzène (Dewailly *et al.*, 2000; Dallaire *et al.*, 2004). Enfin, une étude longitudinale en Espagne a démontré que l'exposition prénatale au DDE peut contribuer au développement de l'asthme à l'âge de quatre ans (Sunyer *et al.*, 2005).

Les organochlorés ont aussi le potentiel de perturber le neurodéveloppement. Certaines études sur des cohortes de naissance ont observé une association entre l'exposition prénatale au DDE et au DDT et les fonctions motrices, cognitives et comportementales des enfants (études revues par Eskenazi *et al.*, 2009). Une étude de Ribas-Fito *et al.* (2007) a mis en évidence un lien entre l'exposition fœtale à l'hexachlorobenzène et un amoindrissement des aptitudes sociales chez les enfants de quatre ans. Un autre organochloré, le mirex, a aussi été identifié en tant que neurotoxique dans une étude où les niveaux dans le placenta étaient associés à une réduction des scores sur des tests de mémoire et d'aptitudes quantitatives à quatre ans (Puertas *et al.*, 2010). Dans une étude sur l'exposition postnatale au DDT et au DDE telle que calculée à partir des concentrations mesurées dans le lait et la durée d'allaitement exclusif et partiel, Pan *et al.* (2009) ont observé un léger effet sur une sous-échelle des fonctions motrices évaluées à l'aide du test Mullen Scales of Early learning administré à l'âge de 12 mois. Cet effet était par contre restreint aux garçons.

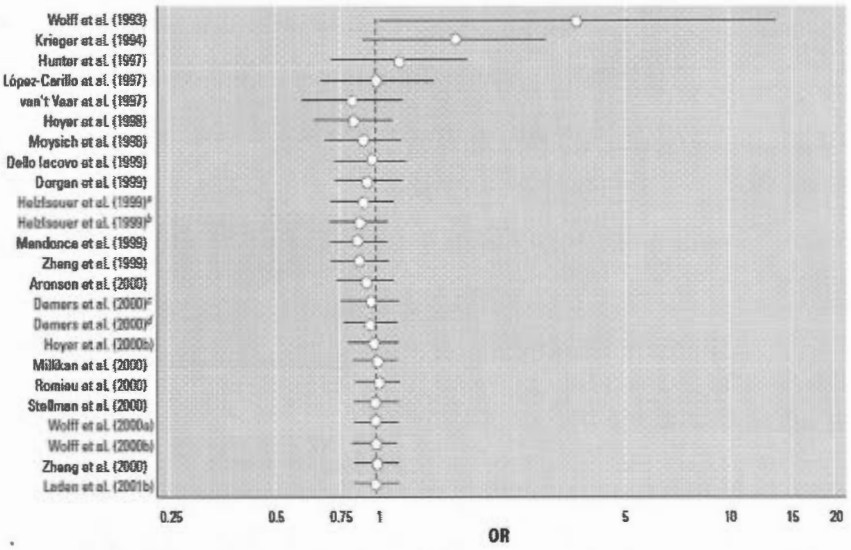

Figure 0.6. Méta-analyse de 24 études cas-témoin sur la relation entre le DDE et le cancer du sein (tiré deLopez-Cervantes *et al.*, 2004).

Alors que quelques études ont rapporté une association entre l'exposition à certains organochlorés et le développement du cancer, la majorité des études publiées jusqu'à maintenant n'ont pas observé de relation significative (Eskenazi *et al.*, 2009). Par exemple, une des premières études sur le cancer du sein publiée par Wolff et al. (1993) portait à croire que l'exposition au DDE augmentait l'incidence du cancer. Par contre, la méta-analyse sur le DDE publiée par Lopez-Cervantes *et al.* (2004) démontre clairement que les résultats des études épidémiologiques sur le cancer du sein qui ont suivi convergent vers une absence d'effet (figure 0.6). Par contre, les études inclues dans cette méta-analyse se sont basées sur des niveaux sanguins en DDE mesurés peu avant ou au moment du diagnostic. Afin d'évaluer les effets d'une exposition plus tôt dans la vie, Cohn *et al.* (2007) ont mesuré le DDT et le DDE dans des échantillons sanguins archivés prélevés chez des femmes américaines enrôlées dans une étude entre 1959 et 1967 alors qu'elles avaient en moyenne 26 ans. Parmi les femmes qui avaient moins de 14 ans en 1945 alors que l'utilisation du DDT était en pleine expansion aux États-Unis, celles étant dans le tertile d'exposition le plus élevé avaient cinq fois plus de chances de développer le cancer du sein que celles se trouvant dans le tertile d'exposition plus faible. Cette étude supporte donc l'hypothèse qu'il existe des fenêtres de susceptibilité pendant ou avant la puberté, une théorie qui n'avait pas été mise à l'épreuve jusqu'alors.

0.1.3. Polybromodiphényléthers (PBDE)

Les PBDE sont des retardateurs de flamme utilisés dans plusieurs produits tels que les articles rembourrés (ex : canapés, matelas), les matériaux de construction, les appareils électroniques et les textiles. La structure chimique de ces composés partage des similarités avec celle des PCB, à

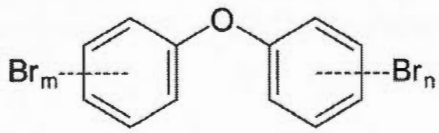

Figure 0.7. Structure des polybromodiphényléthers (PBDE)

l'exception que ces molécules possèdent un pont éther entre les deux anneaux phényliques et que des atomes de brome y sont liés plutôt que des atomes de chlore (figure 0.7). Tout comme les PCB, il existe 209 congénères possibles en fonction du nombre et du positionnement des atomes de brome. Plusieurs études ont documenté la présence des PBDE dans l'environnement et dans les échantillons humains. Alors que l'exposition aux PCB est surtout liée à la consommation de nourriture contaminée, les PBDE se retrouvent dans la poussière à l'intérieur des maisons et sont absorbés lors d'un contact avec celle-ci. Lorber (2008) a estimé le pourcentage de l'exposition attribuable à la poussière à 82 % chez les adultes. Une corrélation a d'ailleurs été établie entre les niveaux en PBDE dans la poussière d'une maison et les niveaux sanguins des résidents (Frederiksen *et al.*, 2010; Johnson *et al.*, 2010). Quant à la toxicité des PBDE, les études publiées jusqu'à maintenant démontrent qu'une exposition à ces molécules peut entraîner une myriade d'effets indésirables dans des modèles *in vitro*, chez les rongeurs et chez l'humain.

Les PBDE et leurs métabolites ont la capacité de perturber le système endocrinien, entre autre par leurs actions agonistes et antagonistes sur les récepteurs des androgènes, des oestrogènes, de la progestérone et de l'hormone thyroïdienne T3 (Hamers *et al.*, 2006). De plus, certains congénères peuvent déplacer la T4 de son site de liaison sur la protéine de transport, la transthyrétine (Hamers *et al.*, 2006). Chez le rat, les PBDE peuvent affecter le système reproducteur en réduisant la synthèse de spermatozoïdes chez les mâles et la synthèse de follicules chez les femelles, par exemple (études revues par Costa *et al.*, 2008). Les expositions

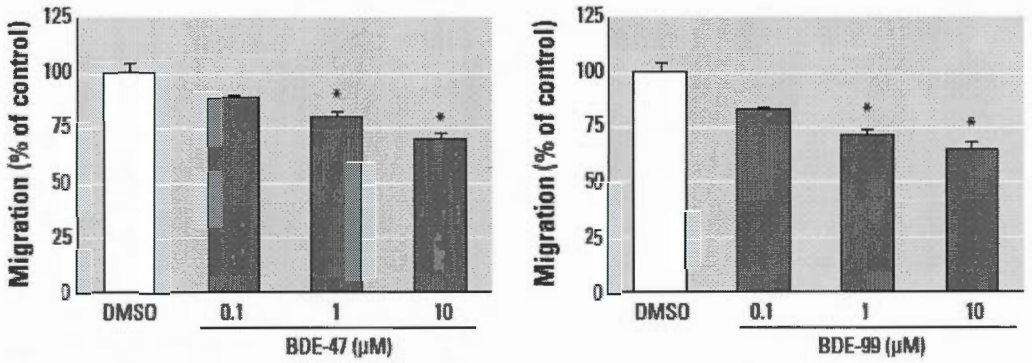

Figure 0.8 Diminution de la migration des cellules progénitrices neuronales humaines par les PBDE-47 et PBDE-99 (tiré de Schreiber *et al.*, 2010).

in vivo chez le rat peuvent aussi entraîner une baisse dans les niveaux en T4 et en œstradiol (Costa *et al.*, 2008). D'autre part, les études chez les souris et les rats ont démontré que l'exposition prénatale aux PBDE peut perturber le neurodéveloppement, notamment en augmentant les comportements d'activité spontanée et en réduisant les aptitudes motrices et la mémoire (études revues par Costa et Giordano, 2007). Des atteintes au développement du cerveau ont été aussi observées dans des modèles *in vitro*. Dans une étude sur des cellules progénitrices du cerveau humain, le PBDE-47 et le PBDE-99 ont entraîné une réduction dans la migration des cellules (voir figure 0.8) et dans la différenciation en neurones et en oligodendrocytes, ces derniers étant responsables de la myélinisation dans le système nerveux central (Schreiber *et al.*, 2010). Les expériences sur des lignées cellulaires neuronales humaines exposées aux PBDE ont démontré plusieurs effets comme l'apoptose (Madia *et al.*, 2004), les dommages à l'ADN et la production de radicaux libres (He *et al.*, 2008). Par ailleurs, Tagliaferri *et al.* (2010) ont démontré que deux congénères, le PBDE-47 et le PBDE-99, peuvent avoir une action synergique sur la production de radicaux libres dans des cellules de neuroblastome humain.

Quelques études chez l'humain ont rapporté des effets des PBDE sur les systèmes reproducteur, endocrinien et neuronal. Chez l'humain, l'exposition prénatale a été corrélée avec la cryptorchidie, soit l'absence d'un ou des deux testicules dans le scrotum (Main *et al.*, 2007). La motilité des spermatozoïdes des hommes inclus dans une étude d'Abdelouahad *et al.* (2011) était diminuée par l'exposition aux PBDE. Harley *et al.* (2010) ont rapporté qu'une exposition aux

PBDE peut réduire la fécondité des femmes, ce qui se traduit en une plus longue période avant de tomber enceinte. Ces altérations du système reproducteur sont possiblement en lien avec la capacité des PBDE à perturber le système endocrinien. Les PBDE dans le sang des femmes enceintes ont été associés à une réduction dans les niveaux en TSH (Chevrier *et al.*, 2010). Dans des échantillons de sang au cordon, certains PBDE ont été associés à une réduction des niveaux en T4 (Herbstman *et al.*, 2008) et en T3 (Lin *et al.*, 2011). Par contre, les résultats d'une étude chez des enfants de quatre ans laissent croire que la perturbation des hormones thyroïdiennes entraînée par une exposition prénatale aux PBDE n'est pas persistante (Gascon *et al.*, 2011). Une étude chez des consommateurs de poisson provenant de la région des Grands Lacs a aussi démontré que les PBDE peuvent entraîner une perturbation des hormones thyroïdiennes et de la testostérone (Turyk *et al.*, 2008).

En ce qui a trait au neurodéveloppement, les trois études épidémiologiques publiées jusqu'à maintenant ont rapporté une association entre l'exposition prénatale et des déficits cognitifs, moteurs ou comportementaux. Dans l'étude de Roze *et al.* (2009) sur un petit échantillon d'enfants de cinq ans (n = 62), les PBDE dans le sang des mères enceintes étaient associés à des déficits de dextérité et d'attention et à de meilleurs scores sur des échelles de coordination, de perception visuelle et de comportement. Aux États-Unis, où les niveaux de PBDE sont beaucoup plus élevés que ceux mesurés en Europe, une réduction dans le développement mental et physique a été observée chez les enfants exposés prénatalement aux PBDE, et ce, de 12 mois à six ans (Herbstman *et al.*, 2010). Alors que les niveaux en PBDE dans le sang au cordon n'étaient pas associés à une altération du neurodéveloppement des enfants dans une cohorte de naissance en Espagne, l'exposition postnatale telle qu'indiquée par les niveaux mesurés dans le sang des enfants à l'âge de 4 ans était associée à une augmentation de la prévalence des symptômes du trouble du déficit de l'attention et à une réduction des aptitudes sociales (Gascon *et al.*, 2011).

Jusqu'à présent, deux études ont été publiées quant à la cancérogénicité des PBDE chez l'humain. Les résultats d'une étude cas-témoin de Hardell *et al.* (2006) sur un petit échantillon (n = 58 cas et 61 témoins) laisse croire qu'une exposition aux PBDE pourrait accroître la prévalence du cancer des testicules. En revanche, l'incidence du cancer du sein ne semble pas

être affectée par l'exposition aux PBDE comme le démontre une autre étude sur 78 cas et 56 témoins (Hurley *et al.*, 2011). Des études de plus grande envergure permettront d'infirmer ou de confirmer ces résultats de façon plus robuste.

0.2. Évaluation de l'exposition aux POP

Bien caractériser l'exposition aux POP durant les périodes de susceptibilité aux atteintes chimiques est un élément critique dans la détection et la quantification des effets que ces composés peuvent avoir sur la santé humaine. Il est donc important de comprendre les facteurs régissant les niveaux internes en POP afin d'interpréter correctement les niveaux mesurés lors des études épidémiologiques. Lorsqu'un échantillonnage est impossible durant la ou les périodes d'intérêt, que ce soit pour des raisons éthiques ou logistiques, des approches doivent être développées afin d'extraire l'information pertinente des échantillons disponibles. Cette section traitera des paramètres d'importance dans la toxicocinétique des POP et des approches disponibles afin d'estimer l'exposition interne à ces composés, notamment la modélisation pharmacocinétique à base physiologique (PBPK).

0.2.1. Toxicocinétique des POP

La toxicocinétique peut être décortiquée en quatre grands processus dictant les niveaux internes d'un composé suite à une exposition, soit l'absorption, la distribution, le métabolisme et l'excrétion. Le processus d'absorption des POP a principalement été décrit pour la consommation de nourriture contaminée et la consommation de lait maternel dans lequel les POP se partitionnent. Les POP qui se retrouvent dans le tube gastro-intestinal sont absorbés de façon passive dans des proportions se rapprochant de 100 % (McLachlan, 1993, Maruyama *et al.*, 2003).

Une fois absorbés, les POP entrent dans la circulation sanguine et subissent un premier passage hépatique. La circulation sanguine les transportera ensuite aux différents organes dans lesquels ils se partitionneront. Puisque ces molécules sont lipophiles, elles se distribuent en fonction de la composition lipidique des organes. On les retrouve alors principalement stockées dans le tissu adipeux, mais elles se distribuent aussi dans le lait maternel qui a un fort contenu lipidique. Par contre, plusieurs phénomènes peuvent influencer la distribution des POP dans le corps humain. Par exemple, une étude d'Imbeault *et al.* (2002) a démontré que les changements de poids peuvent entraîner une remise en circulation des POP stockés dans le tissu adipeux et ainsi

augmenter les concentrations sanguines. Chez l'enfant, la croissance rapide durant les premières années entraîne une dilution des POP accumulés *in utero* et par l'ingestion de lait maternel contaminé. La distribution des POP est donc un facteur très influent sur les concentrations mesurées dans les échantillons de sang, de tissu ou de lait.

Le métabolisme des POP est limité par deux phénomènes : i) leur distribution dans le tissu adipeux réduit la fraction des POP pouvant accéder aux organes où le métabolisme se produit et ii) les taux de métabolisme sont très lents pour la majorité des POP. Pour ces raisons, la demi-vie de ces molécules dans l'organisme peut être de plusieurs années (Shirai et Kissel, 1996). Des métabolites de plusieurs POP ont toutefois été mesurés dans des échantillons sanguins humains, notamment les métabolites hydroxylés des PCB et des PBDE.

Puisque le métabolisme des POP est très limité, ils sont principalement excrétés sans être biotransformés. Ils peuvent être excrétés dans le lait maternel chez la femme, un phénomène supporté par la présence de ces molécules dans les échantillons de lait (Hooper, 1999) et par la réduction dans les niveaux mesurés dans le lait au cours des mois d'allaitement (Thomsen *et al.*, 2010). Ils peuvent également être excrétés dans les fèces par diffusion au travers du tube gastro-intestinal (Moser et McLachlan, 2001).

0.2.2. Approches existantes

Différentes approches ont été proposées afin de caractériser la toxicocinétique des POP chez l'humain. Un modèle statistique multivarié a été développé par Ayotte *et al.* (2003) afin d'estimer les niveaux de PCB-153 chez les enfants d'une communauté d'Inuits. Ce modèle, qui incluait la concentration sanguine en PCB-153 chez la mère, la période d'allaitement, et un indice du volume du tissu adipeux chez l'enfant, a prédit 72 % de la variabilité dans les niveaux sanguins de l'enfant à six mois. Une approche semblable a été utilisée par Karmaus *et al.* (2005) pour reconstruire prospectivement et rétrospectivement des concentrations sanguines en PCB et DDE chez la femme. Par contre, ces modèles développés à partir de bases de données ne génèrent pas de profils toxicocinétiques complets et risquent d'être inadéquats pour les

estimations dans d'autres populations où les caractéristiques démographiques sont différentes (la durée d'allaitement, les habitudes alimentaires et la physiologie, par exemple).

L'exposition aux POP chez l'enfant par l'allaitement a aussi été décrite à l'aide de modèles pharmacocinétiques à un compartiment (Lorber et Phillips, 2002, LaKind *et al.*, 2000). Ces modèles ont un avantage sur la modélisation statistique en ce qu'ils permettent d'obtenir des profils toxicocinétiques complets chez l'enfant. Par contre, les modèles rapportés ici se basent sur une mesure de la charge corporelle en POP chez l'enfant à la naissance et une mesure des niveaux de POP dans le lait maternel en tant que variables indépendantes. Cette approche implique donc l'échantillonnage de ces deux médias lors d'une étude épidémiologique et ne permet pas d'estimer la concentration en POP dans les organes cibles.

Des modèles pharmacocinétiques à base physiologique (PBPK) ont été élaborés afin de caractériser la toxicocinétique de certains POP chez l'humain. Gentry *et al.* (2003), Kreuzer *et al.* (1997) et Ayotte *et al.* (1996) ont développé des modèles d'exposition à la dioxine 2,3,7,8-tétrachlorodibenzo-*p*-dioxine (TCDD) chez l'enfant exposé par l'allaitement. D'autres modèles PBPK ont été développés afin de générer des profils d'exposition interne aux POP chez l'adulte (Emond *et al.*, 2005b, Clewell *et al.*, 2004, van der Molen *et al.*, 1996). Alors que ces modèles sont de bons outils pour l'évaluation de l'exposition interne aux POP, aucune approche jusqu'à maintenant n'a été conçue pour une utilisation à large spectre dans les études épidémiologiques, c'est-à-dire des simulations en série automatisées dans lesquelles les paramètres physiologiques sont basés sur les profils de poids et de taille, ainsi que les historiques d'allaitement des individus à l'étude. De plus, la validation de ces modèles (lorsqu'une étape de validation est effectuée) est jusqu'à présent très limitée. Avant la mise en application de la modélisation PBPK en épidémiologie, il est donc nécessaire d'adapter les modèles afin de permettre l'intégration de paramètres physiologiques des sujets à l'étude et, idéalement, de procéder à une validation à grande échelle.

0.2.3. Modélisation pharmacocinétique à base physiologique (PBPK)

La modélisation PBPK est une approche mathématique décrivant les processus d'absorption, de distribution, de métabolisme et d'excrétion d'un composé dans un organisme. Ces modèles permettent de simuler la cinétique d'un composé pour un vaste éventail de niveaux d'expositions, de voies d'entrée dans l'organisme et de paramètres physiologiques comme le poids corporel. De plus, les modèles validés chez l'animal peuvent être extrapolés chez l'humain en ajustant la structure du modèle et les paramètres physiologiques (ex : volume des organes), biochimiques (ex : taux de métabolisme), et physico-chimiques (ex : coefficients de partage tissu:sang). La conception d'un modèle PBPK s'effectue en quatre étapes principales, soit la représentation, la définition des paramètres, la simulation et la validation (figure 0.9). Une étape de calibration peut suivre ces quatre étapes lorsque la validation échoue.

L'étape de représentation vise à décrire l'organisme de façon conceptuelle et fonctionnelle. Le modèle conceptuel est représenté par un réseau de compartiments (représentant des organes ou des groupes d'organes) reliés par la circulation sanguine. En fonction de l'organisme et du composé étudiés, la représentation conceptuelle prendra différentes formes. Le modèle conceptuel se doit de représenter tous les organes qui entrent en jeu dans les voies

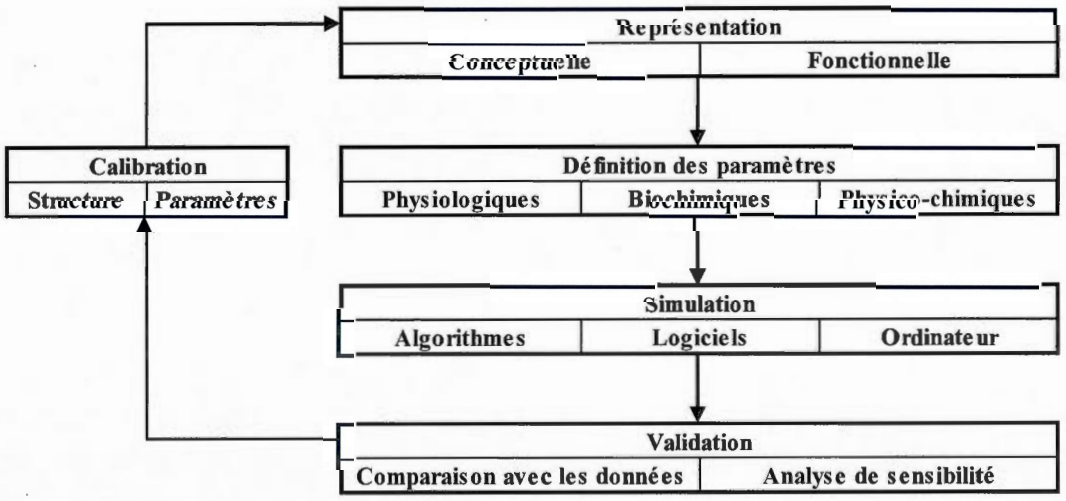

Figure 0.9. Étapes dans la conception d'un modèle PBPK (tiré de Krishnan et Anderson, 2001)

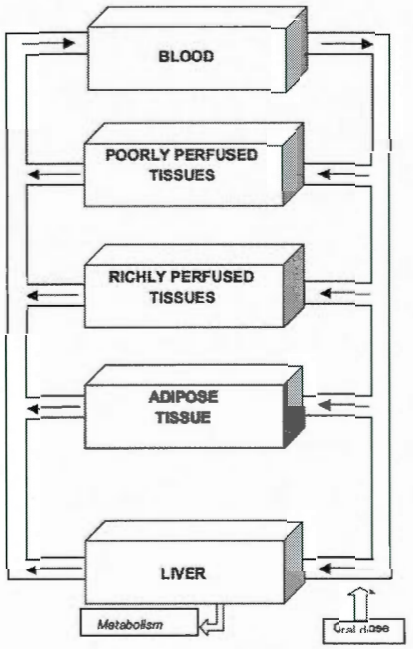

Figure 0.10. Modèle PBPK décrivant la cinétique des PCB chez le rat (tiré de Emond *et al.*, 2005a).

d'exposition, la distribution du composé ainsi que le ou les sites de biotransformation et d'excrétion. De plus, la représentation conceptuelle doit prendre en compte la physico-chimie du composé à l'étude. Prenons, par exemple, un modèle PBPK décrivant la cinétique des PCB chez le rat adulte exposé par voie orale (Figure 0.10). Les PCB sont absorbés dans le tube digestif pour ensuite être transportés jusqu'au foie où a lieu le métabolisme. Cet organe revêt donc une grande importance dans la toxicocinétique des PCB et doit être représenté. Ensuite, le caractère lipophile des PCB fait en sorte qu'ils seront distribués dans les organes en fonction de leur composition lipidique. Les PCB se retrouveront donc principalement dans le tissu adipeux, un compartiment à inclure dans le modèle. La répartition dans les tissus restants sera régit en fonction de la perfusion sanguine des organes qui peuvent être séparés en deux grandes classes : les tissus richement perfusés et les tissus pauvrement perfusés. Des flèches sont utilisées afin de représenter les déplacements du composé par la circulation sanguine entre les compartiments, l'entrée des PCB par la voie orale et l'élimination du composé par le métabolisme dans le foie. Une fois la représentation conceptuelle complétée,

les processus toxicocinétiques dans les compartiments doivent être décrits de façon fonctionnelle. Le comportement des composés dans les compartiments repose sur la perfusion sanguine, le coefficient de partage tissu:sang et le métabolisme dans le cas échéant. Ces différents paramètres sont intégrés dans des équations différentielles de bilan de masse telles que décrites plus en détail dans le chapitre I.

Les valeurs des paramètres propres au composé et à l'organisme doivent ensuite être définies. Ces paramètres peuvent être classés en trois groupes principaux, soit les paramètres physiologiques, biochimiques et physico-chimiques. Parmi les paramètres physiologiques importants, on compte le volume des organes, la perfusion sanguine des différents organes, la production de lait maternel pour les composés excrétés par cette voie et la ventilation pulmonaire dans le cas des composés absorbés par inhalation. Les paramètres physico-chimiques font principalement référence aux coefficients de partage entre deux milieux, incluant les coefficients tissu:sang, sang:air et lait:tissu. Ces coefficients peuvent être déterminés dans des expériences *in vivo* chez les animaux expérimentaux où les niveaux dans les différents média sont mesurés directement, des expériences de partition *in vitro* ou par estimation *in silico* basée sur la composition des différents milieux et les propriétés de la molécule étudiée. Cette dernière approche est particulièrement intéressante dans le cas des POP qui ne se distribuent que dans les lipides; une simple analyse de la composition lipidique des milieux permet d'estimer la partition sans avoir recours à des expériences (Haddad *et al.*, 2000). Enfin, les interactions biochimiques entre les composés et les différentes composantes de l'organisme doivent être caractérisées. Les paramètres biochimiques englobent, entre autres, la liaison du composé à des protéines de transport et les constantes métaboliques et d'absorption.

Suite à la représentation et à la détermination des valeurs des paramètres, il est possible d'obtenir des profils toxicocinétiques suivant une exposition. À cette fin, un algorithme d'intégration doit être sélectionné pour résoudre les équations différentielles de bilan de masse décrivant le comportement du composé dans l'organisme. Plusieurs méthodes d'intégration existent et le choix d'une méthode plutôt qu'une autre peut influencer la précision des simulations et le temps requis pour effectuer ces dernières. Les équations

doivent être codées dans un langage de simulation à l'aide de logiciels informatiques qui pourront ensuite générer des profils toxicocinétiques.

Afin de déterminer la validité du modèle, les résultats des simulations obtenues peuvent être comparés à des données expérimentales. Cette comparaison peut être faite de façon visuelle ou par analyse statistique. Advenant le cas où les résultats obtenus ne concordent pas avec les données expérimentales, une calibration du modèle peut être effectuée en modifiant les paramètres ou la structure du modèle.

OBJECTIFS

Tel que mentionné précédemment, les études épidémiologiques ne réussissent pas toujours à reproduire les effets des POP observés dans des modèles *in vitro* et *in vivo*. C'est le cas, notammant, des études sur le cancer du sein et sur le neurodéveloppement suite à une exposition par l'allaitement. L'évaluation de l'exposition dans ces études est souvent limitée à un échantillon de sang ou de lait maternel. La question à savoir si ces estimations de l'exposition peuvent rendre compte de l'exposition durant des périodes de susceptibilité aux atteintes chimiques demeure entière. Il apparaît donc possible que cette lacune méthodologique soit à la source de l'incapacité des études à observer des associations entre l'exposition aux POP et la morbidité. Afin de réduire l'incertitude quant à l'évaluation de l'exposition durant des périodes critiques de développement, le projet présenté ici visait à :

1) Développer des modèles PBPK qui permettent d'estimer l'exposition interne aux POP durant différentes périodes dans les études épidémiologiques.

2) Appliquer ces modèles au sein d'études épidémiologiques.

Aux fins des études épidémiologiques sur le cancer du sein, un modèle a été développé pour évaluer l'exposition sur toute la durée de la vie des femmes (chapitre I). Ce modèle a ensuite été utilisé afin d'évaluer la capacité d'un échantillon prélevé au moment du diagnostic du cancer du sein à représenter l'exposition durant différentes périodes de la vie de la femme comme la puberté (chapitre II). L'application de ce modèle pour l'évaluation de l'exposition aux PCB dans une étude cas-témoins est en cours et ne sera donc pas détaillée dans cette thèse.

Quant aux études sur l'exposition aux POP par l'allaitement, un modèle décrivant le transfert mère-enfant a été mis sur pied et validé afin de tracer des profils d'exposition postnatale chez les enfants (chapitre III). Ce modèle PBPK a été mis en application dans une étude longitudinale de naissance pour évaluer les effets d'une exposition postnatale aux PCB sur l'attention et l'activité spontanée des enfants inuits âgés de 11 mois (chapitre IV).

CHAPITRE I

PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING OF PERSISTENT ORGANIC POLLUTANTS FOR LIFETIME EXPOSURE ASSESSMENT: A NEW TOOL IN BREAST CANCER EPIDEMIOLOGICAL STUDIES

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Abstract

Background: Despite experimental evidence, most epidemiologic studies to date have not supported an association between exposure to persistent organic pollutants (POPs) and breast cancer incidence in humans. This may be attributable to difficulties in estimating blood/tissue POP concentration at critical time periods of carcinogenesis.
Objectives: In this work we aimed to develop a tool to estimate lifetime POP blood/tissue exposure and levels during any hypothesized time window of susceptibility in breast cancer development.
Methods: We developed a physiologically-based pharmacokinetic (PBPK) model that can account for any given physiological lifetime history. Using data on pregnancies, height, weight, and age, the model estimates the values of physiological parameters (e.g., organ volume, composition and blood flow) throughout a woman's entire life. We assessed the lifetime toxicokinetic profile (LTP) for various exposure scenarios and physiological factors (i.e., breast milk drinking, growth, pregnancy, lactation and weight changes).
Results: Simulations for three POPs [hexachlorobenzene, polychlorinated biphenyl (PCB)-153, PCB180] using different lifetime physiological profiles showed that the same blood concentration at 55 years old can be reached despite totally different LTP. Aside from exposure levels, lactation periods and weight profile history were shown to be the factors that had the greatest impact on the LTP.
Conclusions: This new lifetime PBPK model, which showed the limitations of using a single sample value obtained around the time of diagnosis for lifetime exposure assessment, will enable researchers conducting environmental epidemiology studies to reduce uncertainty linked to past POP exposure estimation and to consider exposure during time windows that are hypothesized to be mechanistically critical in carcinogenesis.

Keywords: Breast cancer, epidemiology, exposure assessment, persistent organic pollutants, physiologically-based pharmacokinetic modeling.

1.1 Introduction

Exposure to ubiquitous persistent organic pollutants (POPs), such as polychlorinated biphenyls (PCB), dichlorodiphenyldichloroethylene (DDE) and hexachlorobenzene (HCB), has attracted attention in breast cancer etiology. These compounds have high solubility in lipids and long half-lives in organisms, and are present in measurable amounts in human tissues, blood and milk. Although the mechanistic actions of these chemicals in carcinogenesis remain unclear, studies showed that some POPs have the potential to promote cancer development in various experimental models such as rodents and human cell lines. *In vitro* assays using MCF-7 human breast cancer cells showed that POPs can promote cell proliferation (Andersson *et al.*, 1999; Du *et al.*, 2000; Soto *et al.*, 1995). POPs have also been shown to inhibit epidermal growth factor withdrawal-induced apoptosis (Davis *et al.*, 2001).

Despite the experimental evidence, controversy still exists regarding breast carcinogenic properties of POP in humans. An epidemiologic study first suggested that DDE blood concentration may be an important etiologic factor in breast cancer (Wolff *et al.*, 1993). This finding led epidemiologists to address further the issue of environmental exposure to POPs and their potential implication in breast cancer. In subsequent years, several environmental epidemiology studies on the subject were published and their findings were greatly variable. Reviews and meta-analysis concluded, on the lack of evidence to support the hypothesis, that POP exposure could be linked to an increase in female breast cancer risk (Calle *et al.*, 2002; Laden *et al.*, 2001; Lopez-Cervantes *et al.*, 2004). On the other hand, some studies showed a positive correlation between POP levels and breast cancer incidence (Aronson *et al.*, 2000; Charlier *et al.*, 2003; Demers *et al.*, 2002; Hoyer *et al.*, 2000; Romieu *et al.*, 2000).

The variability in conclusions among epidemiologic studies might arise from methodologic challenges. One conclusion relates to the lack of tools for past exposure to pollutants assessment (Brody et Rudel, 2003). In most cases, biologic assessment of exposure has been limited to measurements of blood or tissue levels in samples collected around the date of diagnosis. It is uncertain that blood or tissue concentrations sampled a few years before diagnosis reflect the body burden during potentially critical time-windows, such as fetal,

neonatal and pubertal periods. Epidemiologic conclusions on the link between POP exposure and breast cancer development are still to this day based on the premise that single sampling is indicative of past POP exposures, highlighting the need for tools to assess lifetime toxicokinetic profiles.

Physiologically-based pharmacokinetic (PBPK) modeling represents a possible approach to estimate POP exposure during specific time-windows. PBPK models are mathematic representations of xenobiotic pharmacokinetics (i.e., processes of absorption, distribution, metabolism, and excretion) based on the physiologic and biochemical parameters of a given organism (e.g., humans) and the physicochemical properties of the selected xenobiotic (Krishnan et Andersen, 2001). Such models allow the prediction of blood or tissue concentrations at a given time after a given dose of the xenobiotic. Various types of physiologic changes, which can be mathematically described within a PBPK model, may impact the kinetics of a compound in an individual throughout his or her life. Examples of relevant physiological changes are body weight variations, excretion of POPs through lactation (Neville *et al.*, 1991), and physiologic changes due to aging (Haddad *et al.*, 2006; Price *et al.*, 2003a; Price *et al.*, 2003b) or pregnancy (Gentry *et al.*, 2002, 2003). PBPK modeling can also accommodate a variety of exposure scenarios such as changes in the lifestyle of the subject and geographical/temporal monitoring data on environmental levels of POPs. Thus, development of a PBPK model able to simulate exposure throughout life while considering such physiologic changes would be very useful to assess past exposure during critical time windows.

In the past, several PBPK modeling efforts have described the toxicokinetics of different POPs. Many of these models are based on the assumption that these chemicals, which are highly lipophilic, are distributed uniformly between blood and tissues according to their contents in lipids (PCBs: Anderson *et al.*, 1977; Lutz *et al.*, 1977, Lutz *et al.*, 1984; Tuey et Matthews, 1977, 1980a, 1980b; Emond *et al.*, 2005a; Dioxins: Carrier, 1991, van der Molen *et al.*, 1996,Maruyama *et al.*, 2003; Hexachlorobenzene:Yesair *et al.*, 1986). Other models have added diffusion limitation to the fat compartment [Parham et Portier, 1998 (PCBs); You *et al.*, 1999 (p,p'-DDE); Lee *et al.*, 2002, Lee *et al.*, 2007 (PCB153); Belfiore *et al.*, 2007

(Mirex)] or diffusion limitation between erythrocytes and plasma [Lu *et al.*, 2006 (HCB)] to their model structure to improve model predictions of animal kinetic data. Although the addition of diffusion limitation has definitely had an impact on the initial uptake phase on a short time scale, it is not likely to be an important determinant for the toxicokinetics on a scale spanning many years. Most agree that partitioning for these compounds is driven by lipid solubility, and this has been corroborated with human and rodent *in vivo* data (Haddad *et al.*, 2000).

Our overall goal in this work was to develop an exposure assessment tool that could be used in breast cancer epidemiologic studies to estimate lifetime POP blood/tissue exposure and levels during any hypothesized time window of susceptibility in breast cancer development. The specific objectives were to build a generic PBPK model framework to simulate POP toxicokinetics for any given physiological profile and exposure data, and to evaluate through model simulation the impact of exposure scenarios and different physiologic factors such as pregnancy, lactation and body weight on the lifetime internal exposure profile and the blood POP concentration at 55 years of age, a surrogate time representing the age of diagnosis. For the purposes of this study, three POPs with half-lives varying from 6 to 27.5 years were chosen to run the simulations: hexachlorobenzene (HCB), 2,2',3,4,4',5,5'-heptachlorobiphenyl (PCB180), and 2,2',4,4',5,5'-hexachlorobiphenyl (PCB153),

1.2 Methods

The development of this new PBPK model framework for lifetime POP exposure in women can be separated into 3 distinct phases: model representation, model parameterization, and simulations. Model validation could not be achieved because of the evident lack of data – namely a lifetime follow-up study measuring blood concentration at different moments and controlling all the input parameters. The model was coded using Advanced Continuous Simulation Language (ACSLXtreme, Aegis Technologies Group, Inc., Huntsville, AL, USA) and is available on request.

1.2.1 Model representation

For this model, the woman is best functionally described as a network of 9 tissue compartments perfused by blood circulation (Figure 1.1). Compartments are chosen for their known relevance to POP kinetics or in light of objectives of this study. The liver is set as the compartment where both intake via ingestion and metabolism (i.e., first pass organ) take place. Because POPs are highly hydrophobic compounds, adipose tissue is defined as a compartment where significant chemical storage can occur. Because mammary tissue is the cancer site studied and is also an excretory organ (i.e., via lactation), it is also described as a separate compartment. Along with mammary and adipose tissues, uterine tissue, placenta and fetus are represented as compartments because of their variations during pregnancy and post-partum periods (i.e., mainly volume change). The brain is added as a potentially interesting organ for the study of cognitive and motor effects of POPs. Finally, remaining tissues or organs, except for teguments (i.e., bones, nails, hair, cartilage), are grouped into slowly perfused tissues (mainly the skin, skeletal muscles and heart) and richly perfused tissues based on their volume:blood flow ratio.

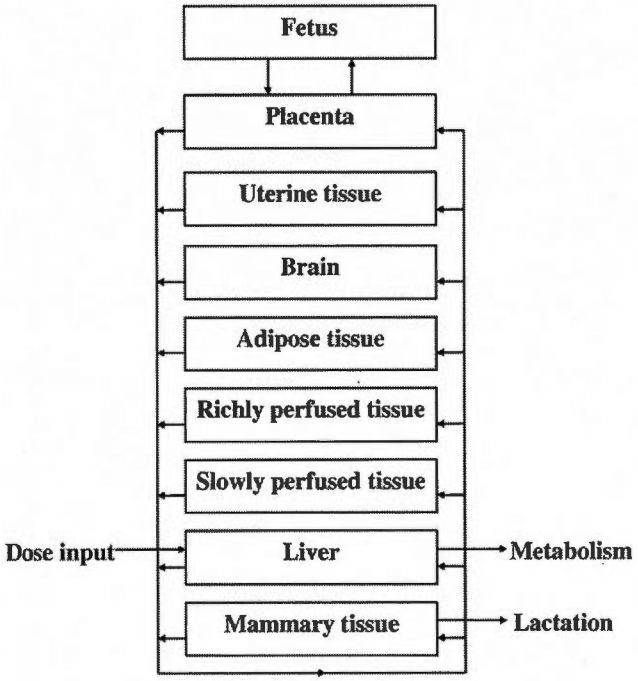

Figure 1.1. Conceptual representation of the PBPK model

Absorption

Absorption of POPs can occur through different routes, but primarily occurs through food intake. For the purpose of this study, total intake is limited to a direct input into the liver compartment, and each POP is assumed to be completely bioavailable through the gastro-intestinal tract, thus involving a hepatic first-pass.

Distribution

The distribution of POPs is managed by the blood flow to the different compartments and partitioning from blood to tissues. This process is described by using mass balance differential equations (MBDEs) that assume homogenous distributions in tissues, as follows:

$$\frac{dAt}{dt} = Qt \cdot \left(Ca - \frac{Ct}{P_{tb}} \right) \qquad [1]$$

where At represents the amount of chemical in the compartment, Qt is the blood flow perfusing the compartment, $P_{t:b}$ is the tissue:blood coefficient for the compartment, Ca and Ct are concentrations in arterial blood and the tissue, respectively.

Metabolism

Metabolism is assumed to be essentially limited to the liver compartment, and the rate is described by the product of the hepatic extraction ratio (Eh), the liver blood flow (Ql) and the arterial blood concentration (Ca) entering the compartment, as follows:

$$RAM = Ql \cdot Eh \cdot Ca \qquad [2]$$

The value of Eh can be calculated from available data such as half-live values, Michealis-Menten constants (Vmax and Km), or intrinsic clearances. The MBDE in the liver therefore becomes:

$$\frac{dAl}{dt} = Ql \cdot (Ca - \frac{Cl}{P_{l:blood}}) - RAM \qquad [3]$$

where Al, Cl and $P_{l:blood}$ are the amount, concentration and tissue:blood partition coefficient of the POPs in the liver, respectively.

Excretion

Because most POPs are poorly metabolized, the main elimination route occurs through excretion of unchanged chemicals. The model is adapted for two excretion pathways: lactation and parturition. POP excretion via lactation is represented as an output from the mammary tissue compartment through a partitioning process between mammary tissue and milk, and milk withdrawal by the suckling such as described by Lee *et al.* (2007) for PCBs in rats. This partitioning process is further addressed in the model parameterization section. The POP excretion via lactation is described as follows:

$$\frac{dAmam}{dt} = Qmam \cdot \left(Ca - \frac{Cmam}{P_{mam:b}} \right) - RAE \qquad [4]$$

and

$$RAE = Qmilk \cdot \frac{Cmam}{P_{mam:b}} \cdot P_{milk:b} \qquad [5]$$

where Amam, and Cmam are the POP amount and concentration in mammary tissue. Qmam is the blood flow to mammary tissue compartment, Qmilk refers to the milk flow out of the breast (i.e., the volume drank per hour by infant in litres per hour), and $P_{mam:b}$ and $P_{milk:b}$ are the mammary:blood and milk:blood partition coefficients.

The placental transfer to the fetus is described using published equations in Gentry *et al.* (2002). The elimination of chemicals through parturition is described as a punctual extraction of the baby body and placenta burdens at the time of birth.

1.2.2 Model parameterization

To simulate internal exposure in women throughout their entire life, compartments size, blood flows and biochemical properties are described as variables that change as a function of age, body weight, body height and pregnancy periods (see Supplemental Material). Mathematic equations describing these variable parameters are arranged so that information on body weight and body height in relation to age, collected from questionnaires, can be easily used as inputs throughout the entire simulation. Volume and blood flow parameters for liver, richly perfused, slowly perfused, and adipose tissue compartments are taken from Haddad *et al.* (2006). The equations describing early stages of development are used to calculate organs growth for the 0-1 year interval because no data are available for that period. Because of the lack of data, uterine tissue and mammary tissue are set as a function of body weight and age (Gentry *et al.*, 2002). Mammary tissue volume is assumed to start from 0 L at the age of 10 and increase linearly to its final volume at 14 years of age.

Physiologic changes during pregnancy are considered for the uterine tissue, mammary tissue, adipose tissue, placenta and fetus compartments based on time elapsed since the beginning of pregnancy, as described by Gentry *et al.* (2002). Postpartum changes are set as a 6-month linear return to normal in the volume of organs influenced by pregnancy (Gentry *et al.*, 2003).

Blood flow to these compartments varies proportionally to their volume throughout pregnancy and postpartum changes. Lactation parameters are also allowed to change over the lactation period. Equations describing changes in daily excreted milk volume and milk lipid content as a function of postnatal time are taken from Neville *et al.* (1991) (see Supplemental Material online at http://www.ehponline.org/members/2008/10917/suppl.pdf). Placental diffusion constant (PAF) describing the exchange between the mother and the baby is given an arbitrary value of 1, because the model outputs in the woman (i.e, blood concentrations) are virtually not influenced by this value.

Tissue:blood and milk:blood partition coefficients are estimated using Poulin and Krishnan's (1996) approach based on tissue water and lipid composition (Price *et al.*, 2003a):

$$P_{t:b} = \frac{K_{ow} x Fl_t + Fw_t}{K_{ow} x Fl_b + Fw_b} \qquad [6]$$

where K_{ow} represents n-octanol:water partition coefficient, Fl and Fw stand for the lipid and water fraction, respectively, for either tissue (subscript t) or blood (subscript b). Tissue composition is taken from Price *et al.* (2003a) (see Supplemental Material online at http://www.ehponline.org/members/2008/10917/suppl.pdf). Slowly perfused tissues compartment composition is calculated as scaled lipid and water content of muscles, skin and heart taken from the same paper as follows:

$$F = \frac{\sum_{i=1}^{n} Vi \times Fi}{\sum_{i=1}^{n} Vi} \qquad [7]$$

where F is the fraction of either lipid or water for the whole compartment, whereas Fi and Vi are the fraction of either lipid or water and the volume for organs included in the compartment, respectively. Richly perfused tissues compartment composition is calculated with the same equation [7]. The richly perfused tissues compartment includes the lungs, the kidneys, reproductive organs, spleen, glands, intestinal tract and stomach tissues. The organ volumes (Vi) used for the calculation of this compartment composition are those at 18 years of age calculated with the equations in Haddad et al. (2001). The partition coefficients for

richly and poorly perfused tissues compartments are calculated with Equation 6 using these scaled composition parameters. Because of the lack of data, the richly perfused tissues compartment partition coefficient is used for the mammary tissue, the placenta and the uterine tissue compartments. Because no information is available on fetus compartment composition, we assessed the impact of different partition coefficients on the model outputs. The lack of significant impact of the tissue:blood partitioning for the fetus compartment on the toxicokinetic profile of the mother led us to arbitrarily give it the partition coefficient of the richly perfused tissues compartment.

Metabolism is parameterized from half-life values for the compounds to be studied. This assumes that POP elimination is essentially attributed to hepatic clearance. First, the intrinsic clearance values per kilogram of liver ($CLint_C$) are calculated for the physiologic parameters at the age of half-life [$HL_{(h)}$] measurements:

$$CLint_C = \left(\frac{Eh_{age} \times Ql_{age}}{1 - Eh_{age}} \right) \Big/ Vl_{age} \qquad [8]$$

where $Eh_{age} = CL_{age} / Ql_{age}$ [9]

$$CL_{age} = \left(\ln 2 / HL_{(h)} \right) \times Vd_{age} \qquad [10]$$

$$Vd_{age} = \left(\sum P_{age} \times Vt_{age} \right) + Vb_{age} \qquad [11]$$

where Ql is the blood flow to the liver, Vl is the volume of the liver, CL is the clearance for the studied compound, Vd is the volume of distribution, P is the tissue:blood partition coefficient, Vt is the volume of tissues, and Vb is the volume of blood. The subscript **'age'** means that the parameters are calculated with the physiologic features at the age of individuals sampled for half-life value measurements, whereas the subscript (h) means that the values of half-lives were in hours. Extraction ratios are calculated from $CLint_C$, which is

assumed to be age invariant and from the liver volume and blood flow, which change as a function of age, as follows:

$$Eh = \frac{CLint_C \times Vl}{CLint_C \times Vl + Ql} \qquad [12]$$

The liver weight-adjusted hepatic extraction ratio is used for the calculation of the metabolism rate. Although recent modeling studies (e.g, Clewell *et al.*, 2004) introduced gene ontology data in their model to reflect age variations in intrinsic clearance, this was not considered in the present simulations because of the relative little impact it has shown on lifetime kinetics and on the data interpretation in this study (simulations not shown). If needed in the future, such information can easily be incorporated into the model.

1.2.3 Model simulations

Different scenarios were simulated in order to assess the impact of different physiologic processes or changes that can occur during the lifetime of a woman on the toxicokinetic profile of POPs as well as on the blood concentration at age of diagnosis. The input parameters that were changed for the simulations were breast-feeding in childhood, level of exposure through food intake, body weight and body height in function of age, number of pregnancies, age at child birth(s), lactation periods and chemical properties for the chosen pollutants (log Kow and hepatic extraction ratio). All simulations used as input the normal body weight profile and the height profile depicted in Figure 1.2, unless stated otherwise.

Three pollutants were chosen for the present studies: PCB-180, PCB-153 and HCB, chosen for their relevant concentrations found in human blood and adipose tissue as well as the fact that they differ in their Kow and half-life. The log Kow was 6.72 for PCB153, 7.21 for PCB180, and 5.73 for HCB [ATSDR, 2000; ATSDR, 2002]. Hepatic extraction ratios were calculated as described in the parameterization section from the approximate half-lives of the chemicals, which were 27.5 years for PCB-153, 9.9 years for PCB-180 and 6 years for HCB in humans (To-Figueras *et al.*, 2000;ATSDR, 2000).

Although the actual food consumption levels of these POP declined from the 1970s to the 1990s, it was kept constant for the purpose of the modeling effort. However, the actual levels may be entered as a variable into the model. For all simulation scenarios, the level of exposure through ingestion of contaminated food was set as a background daily exposure of 10 ng/kg of body weight/day (unless specified otherwise).

Impact of maternal milk drinking in childhood

In this first set of simulations, the period of breastfeeding was set to 6 months, with a constant concentration of 2 µg/L in order to compare the kinetics of the three chemicals. The volume of milk ingested was modeled with the same equation used for the breast-feeding periods of the exposed woman. The milk concentrations used in these simulations were arbitrarily chosen and do not necessarily reflect specific actual levels of contaminants, although similar POP milk levels were found in the literature (Dewailly *et al.*, 1996b; Solomon et Weiss, 2002). The purpose is simply to show how breast-feeding in childhood will impact the LTP of a given individual.

Impact of body weight change

For some simulations, the body weight parameter was varied to investigate the influence of adipose tissue volume and its variation throughout the lifetime of a woman on the tissue pollutant concentration. Body weight and body height profiles used in this study are depicted in Figure 1.2. The normal weight and overweight scenarios represented linear increases in weight from 50 kg at the 14 years to 70 and 90 kg, respectively, at 25 years of age. Weight loss scenario followed the overweight profile with a drop from 90 to 70 kg on a 10-year interval between 25 and 35 years of age.

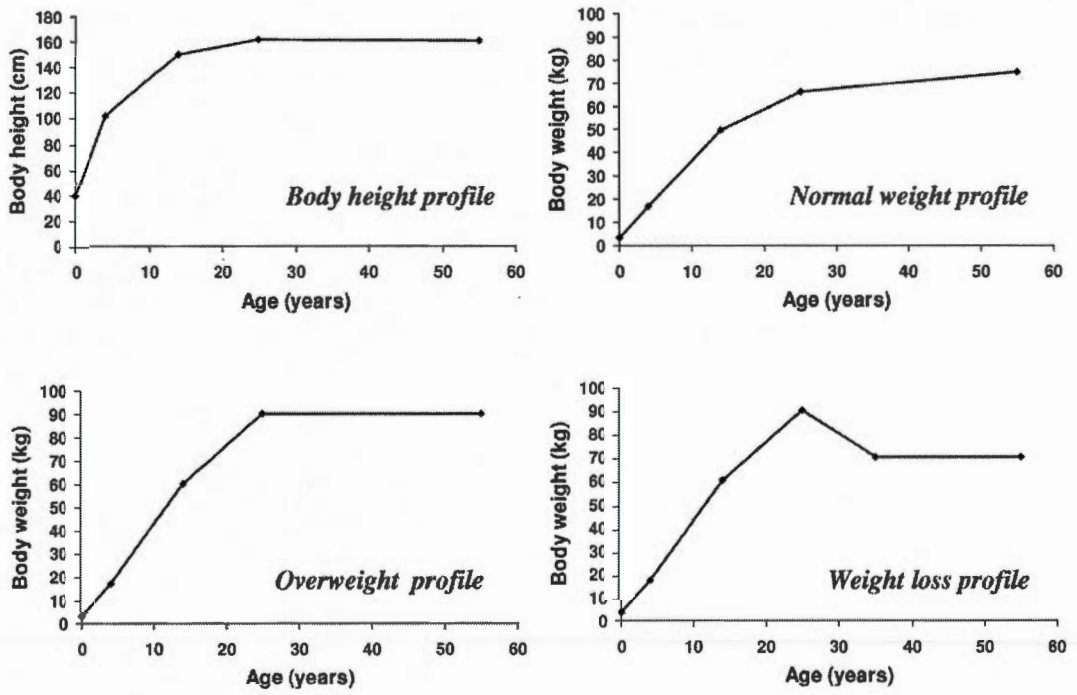

Figure 1.2. Body weight and body height profiles used for the simulations. The normal weight and overweight scenarios represent linear increases in weight from 50 kg at 14 years of age to 70 and 90 kg, respectively, at 25 years of age. Weight loss scenario followed the overweight profile with a drop from 90 to 70 kg on a 10-year interval between the ages of 25 and 35 years.

Impact of pregnancy and lactation

We performed simulations for several pregnancy history scenarios. The number of pregnancies was either one or two. Lactation period length effect on tissue or blood concentration was also assessed; lactation periods chosen for these simulations were 6 and 12 months.

Differences in toxicokinetic profiles for a given blood concentration at the age of diagnosis

For a given POP blood concentration, toxicokinetics profiles were obtained for simulations in women having different physiologic histories (i.e., different number of pregnancies, period of lactation, weight profile and exposure). This was done to assess how much the lifetime internal exposure can differ for a given POP blood concentration at the age of diagnosis. The

exposure values were optimized to reach the same blood concentration at the age of 55 for different physiologic histories.

1.3. Results

1.3.1 Impact of breast-feeding in childhood

We used the PBPK model to investigate the potential impact of additional early exposure through breast-feeding in childhood. For this purpose, a scenario considering breast-milk drinking for a period of 6 months was compared with a scenario where the woman was not breast-fed (Figure 1.3A). The milk concentration was set at 2 μg/L and the background oral exposure was set as a daily intake of 10 ng/kg of body weight. Scenarios were simulated for the normal body weight profile woman. For the breast-fed female scenario, the blood concentration at the end of the milk drinking period reaches 1.87 μg/L for PCB-153, 1.81 μg/L for PCB-180 and 1.74 μg/L for HCB. These concentrations are even higher then those at the age of diagnosis (i.e., 55 years of age). At 5 years of age, the difference between the two scenarios was important for all three pollutants, because the blood concentration was about 2-fold higher for the breast-fed woman. However, by 20 years of age, these differences almost completely disappeared. It can also be observed that blood concentrations at 55 years of age cannot distinguish breast-fed from non-breast-fed individuals, even though internal concentrations in the breast-fed group were very high in infancy.

1.3.2 Impact of body weight change

We also assessed the impact of body weight on POP kinetics with the use of various profiles shown in Figure 1.3. All these simulations used the same body height profile (Figure 1.2). Using these three physiologic profiles, simulations considering a 6-month period of milk drinking in childhood (milk concentration of 2 μg/L) and a daily exposure to 10 ng/kg were performed for the three chemicals studied. Simulations show that both the normal and the overweight scenarios display similar kinetic profiles despite the fact that PCB blood concentration at 55 years of age is slightly higher in the overweight profile than for the normal weight profile. A weight loss between 25 and 35 years of age raises the immediate blood POP concentrations considerably. The effect is more pronounced and lasts up to 55

years of age for PCB-153, where the blood concentrations at 35 years of age are 1.13 µg/L and 1.38 µg/L for the normal weight and weight loss profiles, respectively.

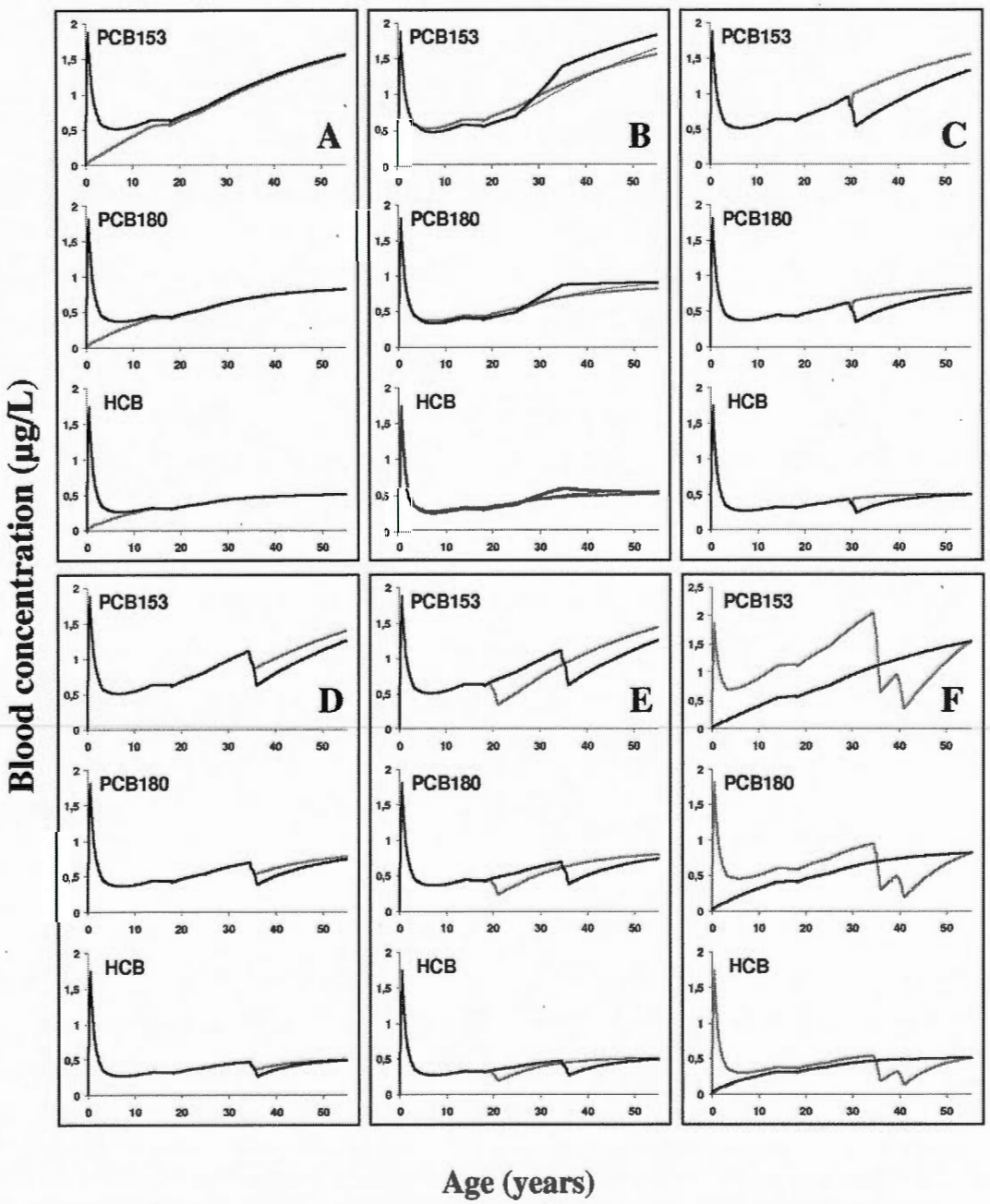

Figure 1.3. Toxicokinetic profiles for PCB153, PCB180 and HCB blood concentration for A) normal body weight history and a 10 ng/kg/day exposure to each of these chemicals for a woman who was not breastfed in childhood (grey line) or breastfed for 6 months (black line); B) normal weight (thin black line), weight loss (bold black line), or overweight (grey line)

profiles and a 10 ng/kg/day exposure to each of these chemicals for women who were breastfed for 6 months in childhood; C) normal body weight history and a 10 ng/kg/day exposure to each of these chemicals for a woman who was breastfed for 6 months in childhood and had a pregnancy at 30 years old followed by no lactation (grey line) or a 12 month lactation period (black line); D) normal body weight history and a 10 ng/kg/day exposure to each of these chemicals for a woman who had a pregnancy at age 35 followed by a 6 month lactation period (grey line) or a 12 month lactation period (black line); E) normal body weight history and a 10 ng/kg/day exposure to each of these chemicals for a woman who was breastfed for 6 months in childhood and had a pregnancy followed by a 12 month lactation period at 20 years old (grey line) or 35 years old (black line); F) normal body weight history for a woman who was exposed to 10 ng/kg/day of each of the three chemicals and had no pregnancy (black line) or was breastfed for 6 months in childhood and was exposed to 18.7 ng/kg/day of PCB153, 13.8 ng/kg/day of PCB180, 11.6 ng/kg/day of HCB, and who had two pregnancies at age 35 and age 40 followed by 12 month lactation periods (grey line).

1.3.3 Impact of pregnancy and lactation

We also investigated the impact of pregnancy and subsequent breast-feeding on blood POP concentrations. First, scenarios with pregnancy alone and pregnancy followed by a 12-month lactation starting at 30 years of age were simulated in order to compare the respective effect of these two factors (Figure 1.3C). For PCB-153, the blood concentration at 31 years of age is much lower in the woman who lactated for 12 months (0.53 μg/L blood) than in the woman without lactation (1.01 μg/L blood). The difference is still present at 55 years of age, although smaller than at 31 years of age. For PCB-180, lactation has a small effect on the blood concentration at 55 years of age, whereas for HCB it has no impact. Simulations show that the pregnancy alone induces a small drop in the blood concentration that rapidly returned to prepregnancy levels after postpartum physiologic changes (Figure 1.3C, normal lines).

We assessed the impact of the length of the lactation period. Simulations for two lactation period lengths show that longer lactations have a greater impact on the blood concentration (Figure 1.3D). For PCB-153, the blood concentration at 55 years of age is 1.39 μg/L for a 6-month lactation and 1.25 μg/L for a 12-month lactation starting at 35 years of age. The difference between the two lactation periods is small for PCB-180 and negligible for HCB when only blood concentration at 55 years of age is considered.

POP toxicokinetic profiles were also compared for lactations of the same duration but held at different ages. Two scenarios were compared: child birth at either 20 or 35 years of age followed by a 12-month breast-feeding period (Figure 1.3E). As expected, POP kinetics differ between the two scenarios. The effect of a lactation period later in life influences significantly the blood concentration at 55 years of age for PCB-153, whereas this effect is minimal for PCB-180 and practically absent for HCB in the simulated scenarios.

1.3.4 Differences in toxicokinetic profiles for a given blood concentration at diagnosis

To demonstrate that a same blood concentration at 55 years of age can be the result of completely different kinetic profiles, we compared two scenarios with different lifetime profiles (Figure 1.3F). The first scenario represents a woman who has not been breast-fed in childhood, was never pregnant and was exposed throughout life to the background level of 10 ng/kg/day for each of the three pollutants. The second scenario is that of a woman who was breast-fed for 6 months with breast milk at a POP concentration of 2 µg/L, and had two pregnancies, one at 35 and one at 40 years of age, followed by a 12-month lactation period each time. To obtain a final concentration at 55 years of age identical to the one in the first scenario, the daily exposure levels were optimized to the following levels: 18.7 ng/kg/day for PCB-153, 13.8 ng/kg/day for PCB-180 and 11.6 ng/kg/day for HCB. These simulations yielded completely different kinetic profiles despite resulting to the indentical blood concentration at 55 years of age (Figure 1.3F). The blood concentration at 34 years of age showed the greatest difference, especially for PCB-153 where approximately a 2-fold higher level was calculated for the breast-feeding/pregnancy/higher exposure scenario (2.03 µg/L blood) than for the no breast-feeding/no pregnancy/background exposure scenario (1.07 µg/L blood).

1.4 Discussion

Over the last decades, many environmental epidemiology studies have focused on the possible link between exposure to POPs and the development of breast cancer, but no clear overall conclusion could be drawn from the different findings. Discrepancies among conclusions from the various studies might be related to the false assumption that a unique late-life sampling reflects lifetime POP exposure. Our study supports this contention and proposes a new tool that could reduce this uncertainty in exposure assessment by simulating lifetime toxicokinetics of POPs in women.

The PBPK model built in this study can overcome exposure assessment problems by simulating normal development (i.e., growth, blood flows) and various historical events within the lifetime of a woman [i.e., breast feeding, changes body mass index (BMI), pregnancy] to obtain the lifetime toxicokinetics of POPs. Using this model, we simulated several exposure and physiologic scenarios to assess the impact of different parameters on POP blood concentrations from 0 to 55 years of age.

Although *in utero* exposure is known to occur through placental diffusion, body burden at birth was set to 0. This methodological choice relies on two facts: a) fetus tissue concentration estimation would require information on the exposure of the mother to simulate the placental transfer, and b) that the baby's body burden at birth is rapidly diluted by increasing tissue volumes and therefore has a small impact on lifetime toxicokinetics when compared to breast milk consumption (Clewell *et al.*, 2004; Kreuzer *et al.*, 1997).

Simulations showed that although pregnancy alone did not have a strong impact on blood POP concentrations, lactation exerted major changes in the toxicokinetic profiles. The longer and the later in life a lactation period occurs, the greater its impact on blood POP concentration of the woman at 55 years of age. Thus, quantitative information on lactation is critical when evaluating past exposure to POPs. Moreover, simulations showed that body weight variations through life seemed to have a greater impact on blood POP concentrations than body weight level itself. A loss of weight can be regarded as a decrease in the adipose

tissue volume in which POPs are preferentially stored, a phenomenon that leads to the unloading of POPs into blood. It has been previously shown that PCB-153, HCB, β-hexachlorocyclohexane, *p,p'*-DDE and Aroclor 1260 levels in blood increase with weight loss (Imbeault *et al.*, 2002). Therefore, BMI changes should be regarded as an important factor in POP kinetics. New approaches in exposure assessment that consider physiologic parameters such as BMI and lactation were developed by Wolff *et al.* (2005) with the use of first-order pharmacokinetic and predictor-based multivariate models. They concluded that possible exposure misclassifications in epidemiological studies can occur if the impacts of BMI and lactation on POP concentrations are ignored. In accordance with such results, the current study clearly showed that sampling at the age of diagnosis is a questionable endpoint for lifetime exposure estimation. A more recent study from Wolff *et al.* (2007) stresses the importance of considering pharmacokinetic variability in epidemiological studies. PBPK modeling as proposed here is particularly well suited for such considerations The use PBPK models, such as the one reported in our manuscript, go much further than Wolff's strategy in considering pharmacokinetics. This new approach is being proposed to epidemiologists. Instead of simply proposing pharmacokinetic factors such as BMI as other covariables in the epidemiologic studies, we suggest directly using PBPK model estimates of blood or tissue concentrations during different periods of life for each subject of the study to analyze if there is a relationship between disease and internal exposure.

We investigated the impact of breast milk consumption in childhood on the internal exposure by comparing the POP venous concentration in a breast-fed and a bottle-fed women. Results showed that early-life blood POP concentrations are strongly influenced by breast-milk drinking for the first years of life, but that these effects are almost fully attenuated by 20 years of age. These findings are supported by a study on a Faroese birth cohort in which the primary contributors to the serum total PCB concentrations at 7 and 14 years of age were breast-feeding and blubber consumption, respectively (Barr *et al.*, 2006). The work reported herein showed that blood or tissue POP concentration at the age of diagnosis does not reflect the important body burden resulting from early-life breast-feeding, a possibly important time window of exposure.

By simulating the lifetime blood concentrations for two distinct exposure and life history scenarios with the same level at 55 years of age, this study showed the poor predictive value of late-life sampling for past exposure assessment. Late lactations can dramatically decrease POP concentrations and lead to lower blood concentrations at the age of diagnosis, even for a high-exposure profile. To eliminate this artifact, it is crucial that the estimation takes into account such physiologic events.

This PBPK model enables the consideration of chemical-specific parameters affecting distribution (log Kow) and elimination (half-life). The simulations performed with the three contaminants showed that blood PCB-153 levels were more sensitive to the main factors—that is, lactation and body weight change—than those for PCB-180 or HCB. This can be explained by their different half-lives, a parameter which strongly correlates with the time required for the pollutant to reach steady state in the body. Chemicals with a shorter half-life reach steady-state more rapidly, leading to the faster attenuation of the impacts that these physiologic events may have on blood concentration. On the other hand, the log Kow is unlikely to account for differences in POP toxicokinetics, because it has been reported that compounds with a log Kow value > 4 will partition similarly between blood and organs (Haddad *et al.*, 2000). Like physiologic parameters, interindividual variability also exists in half-life values as well as blood and adipose tissue lipid content, which are sensitive parameters of the PBPK model (see Supplemental Material online at http://www.ehponline.org/members/2008/10917/suppl.pdf). Apart from doing a toxicokinetic study in each individual there currently exists no method to estimate half-lives in individuals. Similarly, determining lipid fractions in blood and adipose tissue of individuals represents another difficulty and such a practice can be very costly. Using average values of these parameters is an acceptable surrogate, because they lead to prediction errors under a 1.5-fold difference in blood concentrations in the case of intrinsic clearance and 1.8-fold in the case of lipid composition in adipose tissues and blood (see Supplemental Material http://www.ehponline.org/members/2008/10917/suppl.pdf).

The PBPK model developed herein refines the assessment of past tissue exposure by incorporating physiologic processes that greatly affect POP kinetics. The use of such a tool

should permit epidemiologists to better assess past exposure and to investigate the potential critical windows of exposure to POPs in cancer development, a commonly reported concern in epidemiology studies. The importance of exposure assessment for different critical time windows is supported by studies on breast cancer incidence among Japanese women who were exposed to radiation; these studies show that exposure at a lower age has a higher impact on cancer development than exposure at later-life stages (Hoel et Dinse, 1990; Tokunaga *et al.*, 1994). Moreover, a recent study reported that exposure to *p,p'*-DDT early in life may increase the risk of breast cancer (Cohn *et al.*, 2007). By using estimated LTPs and internal exposure levels for different time frames, this hypothesis could be further addressed.

Furthermore, the toxicity of certain POPs may not be attributable entirely to parent compound. Some evidence indicates that metabolites may also elicit a biochemical or toxic response (Safe, 1994; You *et al.*, 2006; Meerts *et al.*, 2004; Machala *et al.*, 2004). Because the PBPK model describes the metabolism of the chemicals, the amount of metabolites formed in different periods of life can be assessed and used for epidemiologic analysis in the same way as we use internal concentrations of the parent compound. Thus, PBPK modeling can also add another dimension to POP epidemiologic studies.

To perform simulations, information on the proposed important variables to be used as inputs in the model must be gathered within the epidemiologic questionnaire. The model was constructed in such a way that it requires information on body weight and height as a function of age. Information on pregnancy and lactation periods is also a prerequisite to model simulation. This information must be associated with the age of the woman at birth of her children and the duration of the lactation periods. The model can easily include any information on exposure levels that could vary as a function of changes in dietary lifestyle (e.g., increase in fish consumption or in fatty foods for certain periods of life) as well as geographic/temporal monitoring data on environmental levels of selected pollutants. Once these parameters are included in the model, the exposure scenario can be optimized with the use of information on both estimated exposure and blood or tissue samples.

Although the proposed PBPK model framework for POP lifetime exposure assessment in women is constructed on validated descriptions of absorption, distribution, metabolism and excretion of various POPs in rodents or humans, further studies are needed to validate the model. The model proposed herein can be used as a generic framework in which adjustments/modifications can be made to incorporate additional toxicokinetic processes that may be specific to particular POPs (e.g., diffusion limitation, plasma or tissue protein binding) if relevant for a lifetime scale in humans.

1.5 Conclusion

This study is the first to propose a PBPK modeling approach for the assessment of lifetime internal exposure to POPs in the context of epidemiologic studies. The proposed model has the potential to be used in environmental epidemiology research to reduce the uncertainty in past tissue exposure estimation. This approach can not only strengthen the validity and reproducibility of studies on the impact of POPs on breast cancer incidence in humans, but also help to assess the effect of exposure during critical time windows in breast cancer development as well as other late-life diagnosis pathologic end points.

Acknowledgments

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Supplemental material

Model parameters

The parameters for the proposed generic PBPK model for POPs were obtained from the literature. The physiological parameters are estimated according to different equations listed in Table 1.S1, they are a function of body weight, height and/or age. The functions are either taken directly from Price *et al.* (2003a) or derived from the same data (Haddad *et al.*, 2006). Physiological changes relating to pregnancy are also considered by using equations from Gentry *et al.* (2002) (Table 1.S1). Parameters related to lactation are also estimated from equations from Neville *et al.* (1991). These show relationships between volume of milk or the milk composition and the number of days postpartum (Table 1.S2).

Blood and tissue composition data needed to determine tissue:blood partition coefficient were also taken from the literature and are shown in Table 1.S3. All values were assumed to be the same throughout life except for adipose tissues and muscle and skin for which data showed significant difference in lipid content between children younger than 14 years old and adults. The values in lipid composition between 14 and 18 years of age were estimated by assuming a linear increase from values at 14 to those at adulthood (i.e., 18 years old).

Table 1.S1. Physiological parameters for organ volumes and blood flows as a function of age, body weight and body height.

Parameters	*Age*	*Equations*
Volumes (L)		
Body surface cm^2 (S)[1]	0-55	$=BW^{0.5150} \times BH^{0.4220} \times 234.9$
Volume of liver (Vl)[1]	0-55	$=0.0501 \times BW^{0.780}$
Volume of richly perfused tissue (Vrp)[1]	0-3	$=(-1.919E\text{-}2 \times Age + 3.193 \times (BW^2/BH)^{0.2657} - 1.374) - Vl$
	3-18	$=(2.515E\text{-}2 \times Age + 7.619 \times (BW^2/BH)^{0.1499} - 6.098) - Vl$
	18-55	$=(2.331E\text{-}3 \times Age + 0.1253 \times BW^{0.8477} + BH^{0.3821} - 4.725) - Vl$
Volume of slowly perfused tissue (Vsp)[1]	0-55	=Vtongue + Vheart + Vskmuscles
Volume of tongue (Vtongue)[1]	0-55	$=1.190E\text{-}3 \times BW - 4.302E\text{-}4$
Volume of heart (Vheart)[1]	0-55	$=1.017E\text{-}7 \times (BH^{0.6862} \times BW^{0.3561} \times 242.7)^{1.420}$
Volume of skeletal muscles (Vskmuscles)[1]	0-3	$=9.563E\text{-}2 \times BW + 1.650E\text{-}2 \times BH + 9.102E\text{-}2 \times Age - 1.642E\text{-}1$
	3-18	$=1.629E\text{-}1 \times BW + 2.603E\text{-}2 \times BH + 4.661E\text{-}1 \times Age - 3.332$
	18-55	$=6.780 \times (S/10^4)^{1.629} - 1.492E\text{-}3 \times Age + 3.580$
Volume of skin (Vs)[1]	0-55	=Vdermis + Vepidermis
Volume of dermis (Vdermis)[1]	0-10	$=0.664 \times (S/10^4)$
	10-20	$=-9.356E\text{-}5 - 2.151E\text{-}5 \times Age - 5.058E\text{-}1 \times (S/10^4) + 1.134E\text{-}6 * Age^2 + 0.117 \times Age \times (S/10^4) - 1.673E\text{-}5 \times (S/10^4)^2$
	20-55	$=1.834 \times (S/10^4)$
Volume of epidermis (Vepidermis)[1]	0-55	$=7.850E\text{-}2 \times (S/10^4)^{1.049}$
Volume of fat (Vf)	0-55	=Vf_basal + Vf_Incr + Vf_Decr
Basal volume of fat (Vf_basal)[1]	0-55	$=0.91 \times BW - (Vl + Vr + Vp + Vs)$
Fat volume increase during pregnancy (Vf_Incr)[2]		$=BWx\left(0.09x\,e^{\left[-1.290995862 e^{(-0.000792 Hours\_S)}\right]}\right)$
Extra fat volume at the end of pregnancy (Vf_endP)[3]		$=BW \times 0.084026$
Fat volume decrease post-pregnancy (Vf_Decr)[4]		$=Vf\_endP + (-Vf\_endP \times (Hours\_P/4380))$
Volume of mammary tissue (Vmam)		=Vmam_basal + Vmam_Incr + Vmam_Decr
Basal mammary tissue volume (Vmam_basal)[2]		$=0.0062 \times BW$
Mammary tissue volume increase during pregnancy (Vmam_Incr)[2]		$=BWx\left(0.0065x\,e^{\left[-7.448684732 e^{(-0.000678 Hours\_S)}\right]}\right)$
Extra mammary tissue volume at the end of pregnancy (Vmam_endP)[3]		$=BW \times 0.007088$
Mammary tissue volume decrease post-pregnancy (Vmam_Decr)[5]		$=Vmam\_endP - (Vmam\_endP \times (Hours\_P/4380))$
Volume of uterine tissue (Vu)	0-55	=Vu_basal + Vu_Incr + Vu_Decr

Basal volume of uterine tissue (Vu_basal) [2] Uterine tissue increase during pregnancy (Vu_Increase) [2] Extra volume of uterine Tissue at the end of pregnancy (Vu_endP) [3] Uterine tissue volume decrease post-pregnancy (Vu_Decrease) [5]		=0.0014 x BW $=BWx\left(0.02xe^{\left[-4.715669973e^{(-0.000376xHours\_S)}\right]}\right)$ =BW x 0.029799 =Vu_endP + (-Vu_endP*(Hours_P /4380))
Volume of placental tissue (VPla) [2]		$=0.85xe^{\left[-9.434xe^{(-5.23e-4xHours\_S)}\right]}$
Fetal volume (Vfet) [2]		$=3.50x\left(e^{\left[-16.081xe^{(-5.67e-4xHours\_S)}\right]}+e^{\left[-140.178xe^{(-7.01e-4xHours\_S)}\right]}\right)$
Blood flows (L/H)		
Blood flow to heart (Qc)	0-55	=Qc_basal+ (Qmam-Qmam_basal) + (Qu-Qu_basal) + (Qf-Qf_basal) + QPla
Basal blood flow to heart (Qc-Basal) [1]	0-55	$=15.048 \times BW^{0.7609}$
Blood flow to liver (Ql) [1]	0-55	=60.00 x Vl
Blood flow to richly perfused tissue (Qr) [6]	0-55	=Qc_basal - (Qsp + Qs + Qf_basal + Ql + Qu_basal + Qmam_basal)
Blood flow to slowly perfused tissue (Qsp) [1]	0-55	=1.80 x (Vtongue + Vskeletalmuscle) + 57.60 x Vheart
Blood flow to skin (Qs) [1]	0-55	=9.0 x (Vdermis + Vepidermis)
Blood flow to fat (Qf)	0-55	=Qf_basal x Vf/Vf_basal
Basal blood flow to fat (Qf_basal) [1]	0-55	=1.80 x Vf
Blood flow to mammary tissue (Qmam)	0-55	=Qmam x Vmam/Vmam_basal
Basal blood flow to mammary tissue (Qmam_basal) [2]	0-55	=0.027 x Qc_basal
Blood flow to uterine tissue (Qu)	0-55	=Qu_basal x Vu/Vu_basal
Basal blood flow to uterine tissue (Qu_basal) [2]	0-55	=0.0062 x Qc_basal

Hours_S stands for hours after start of pregnancy
Hours_P stands for hours post-partum
[1] Equation used in Haddad *et al.* (2006)
[2] Equation used in Gentry *et al.* (2002)
[3] Calculated from Gentry *et al.* (2002)
[4] Based on Gentry *et al.* 2003
[5] Equation from present study
[6] Adapted from Haddad *et al.* (2006)

Table 1.S2. Parameters for milk volume and contents as a function of time post-partum (adapted from Neville *et al.*, 1991)

Parameters		***Equations***
Vmilk	Volume of milk (L/H)	=(1.069 – 0.001212 x Days_P)/24
Fl_milk	Lipid content of milk (%)	=3.8 + 0.0095 x Days_P
Fp_milk	Protein content of milk (%)	=0.8 + 0.0004 x Days_P
Fw_milk	Water content of milk (%)	=100 – Fl_milk – Fp_milk

Days_P stands for the number of days post-partum

Table 1.S3. Compartment composition parameters used for partition coefficient calculation (taken from Price *et al.*, 2003a).

		Compartment composition	
Compartment	***Age***	***Fl (%)***	***Fw(%)***
Blood	0-55	0.6	79.0
Brain	0-55	11.6	75.5
Muscles and skin	0-14	2.1	74.1
	14-18	*linear increase to adult values*	
	18-55	4.2	74.1
Heart	0-55	1.0	73.0
Adipose tissue	0-14	55.0	41.1
	14-18	*linear increase to adult values*	
	18-55	74.1	21.2
Richly perfused[1]	0-55	3.68	78.1

[1] Values calculated as described in manuscript. These composition values were also used to for the mammary, placenta, fetus and uterine tissues.

Sensitivity analysis

A sensitivity analysis was made to determine parameters most influent on blood concentrations over lifetime (AUCblood) at different ages (i.e., 5, 14, 25, 40 and 55 years old). Figure 1.S1 displays the relative sensitivity coefficient of each parameter that is independent of BW, BH and/or age. The simulation used a PCB 153 exposure scenario with breast milk drinking in the first year of life and a single birth during adulthood with a constant environmental exposure of 20 ng/kg/day. The parameters relating to distribution in adipose tissue (Fla, Flc and Flb) were most sensitive followed by intrinsic clearance (Clint). Other parameters that were obviously influent during adulthood were those that touched excretion through breast-feeding: Duration (MDDUR) and lipid content of milk (Fmlki). The age at which the mother has her baby (Agebaby1) also had an impact only at 40 years of age. The impact of the duration of the breast milk drinking after birth was great in early life and diminishes as age increases.

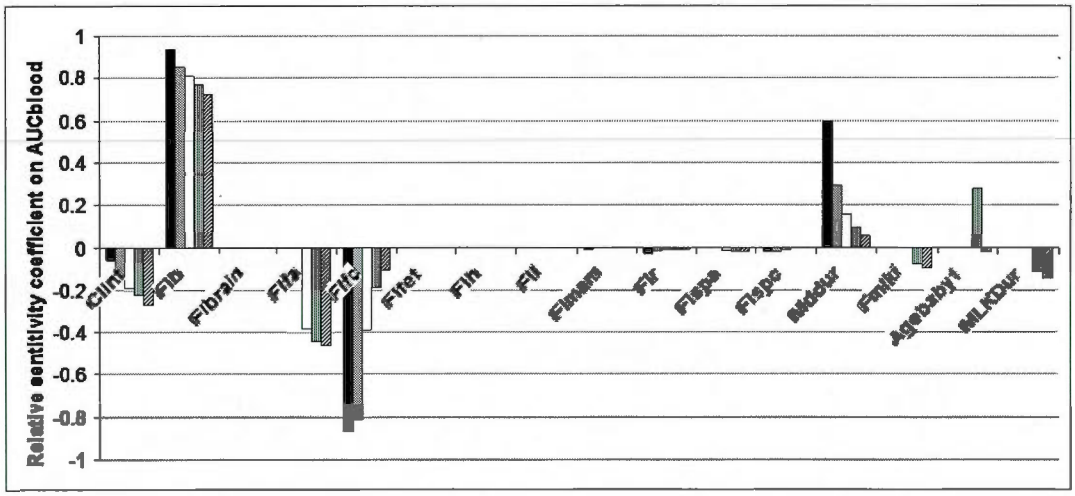

Figure 1.S1. Analysis of parameter sensitivity for different model parameters on the AUCblood at different ages (black= 5 years, gray= 14 years, white= 25 years; vertical line= 40 years, and diagonal lines= 55 years). Clint=intrinsic clearance; Flb=lipid fraction in blood; Flbrain= lipid fraction in brain; Flfa= lipid fraction in adult fat; Flfc= lipid fraction in children fat; Flfet= lipid fraction in feotus; Flh lipid fraction in heart= ; Fll= lipid fraction in liver ; Flmam= lipid fraction inmmmay tissues; Flr= lipid fraction in richly perfused tissues ; Flspa= lipid fraction in adult slowly perfused tissues , FLspc= lipid fraction in children slowly perused tissus; Mddur= duration of breast milk drinking in infancy; Fmlki= lipid

fraction in milk; Agebaby1= age of mother at first pregnancy; MLKDur= duration of breastfeeding after pregnancy.

Variability

Because all physiological parameters change as a function of age, BW and/or BH, it was not possible to include them in the sensitivity analysis performed in the previous section. Therefore, different Monte Carlo (MC) simulations were performed to determine the impact of variability of BW, BH and sensitive parameters on the blood concentration (Cblood) vs time profile.

Impact of BH, BW on toxicokinetic profile

Using data derived from distribution charts of BH vs age (Figure 1.S2), BMI vs age (Figure 1.S3) and BH vs BW (Figure 1.S4) for american women (CDC 2008 and Halls.md 2008), distributions of Cblood vs time profiles were performed. In each run of the MC, the subject's baseline BH and BMI percentile was randomly selected (from 5^{th} to 95^{th}). The percentile of BMI was randomly modified by up to ±5% for each of four periods as the subject aged. For BW, an initial percentile was first determined and initial values were determined from the BH vs BW chart according to percentile chosen and BH value previously determined (Figure 1.S4). At ages above 2, the BW is then estimated by using available BMI distribution values for women from 2 to 55 years old, as follows:

$$BW = BMI * (BH/100)^2 \qquad \text{Equation S1}$$

The BMI percentile value was allowed to increase or decrease during life with change up to a maximum of 20%. The Figure 1.S5 shows the impact of variability in BW and BH in a population on Cblood for a given exposure and life scenario. The variability in blood concentrations introduced by BH and BW is greatest in infants after birth (i.e, variations up to 2-fold) and after lactation (i.e., variations up to 3-fold) in the population. There is a variability of about 1.3 for the rest of life.

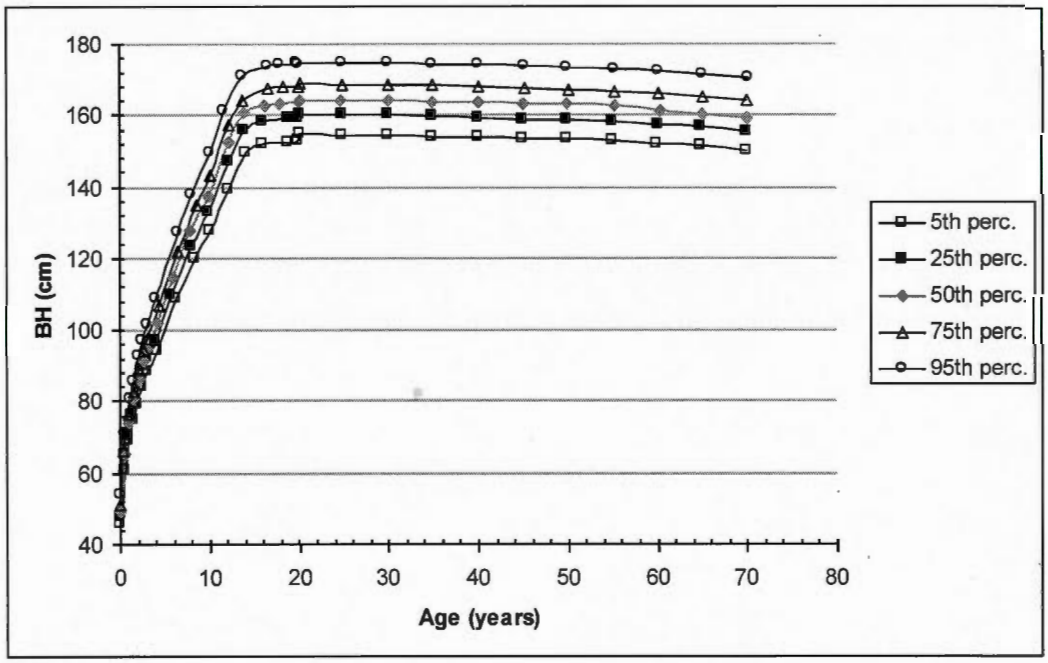

Figure 1.S2. Female growth chart. Symbols represent extracted data (from CDC, 2007 and Halls.md, 2007) used for interpolation in the PBPK model.

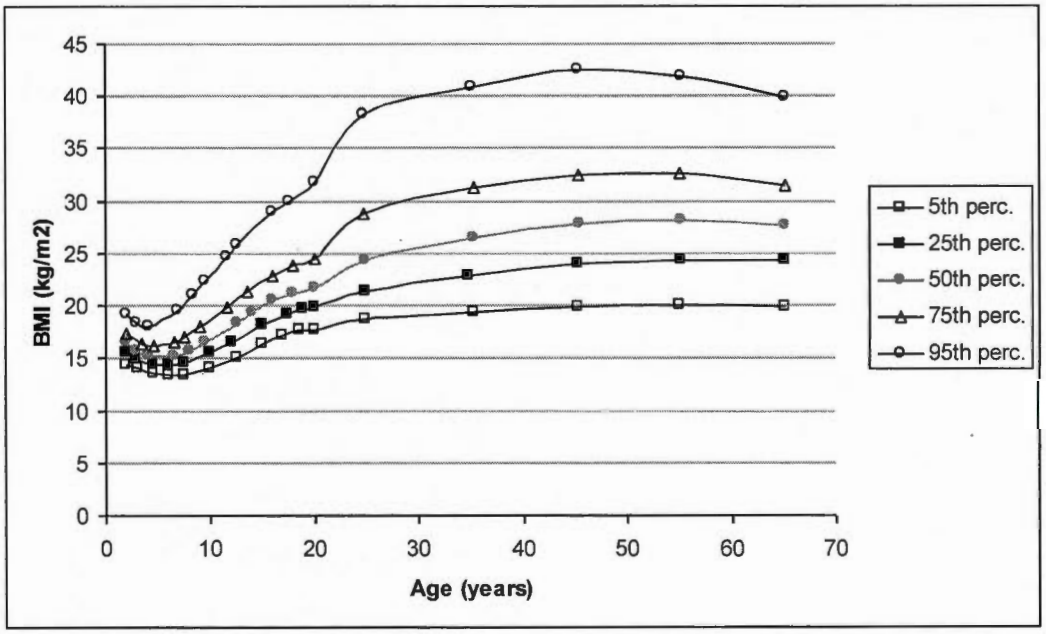

Figure 1.S3. BMI chart in function of age for females of 2 to 65 years old. Symbols represent extracted data (from CDC, 2007 and Halls.md, 2007) used for interpolation in the PBPK model

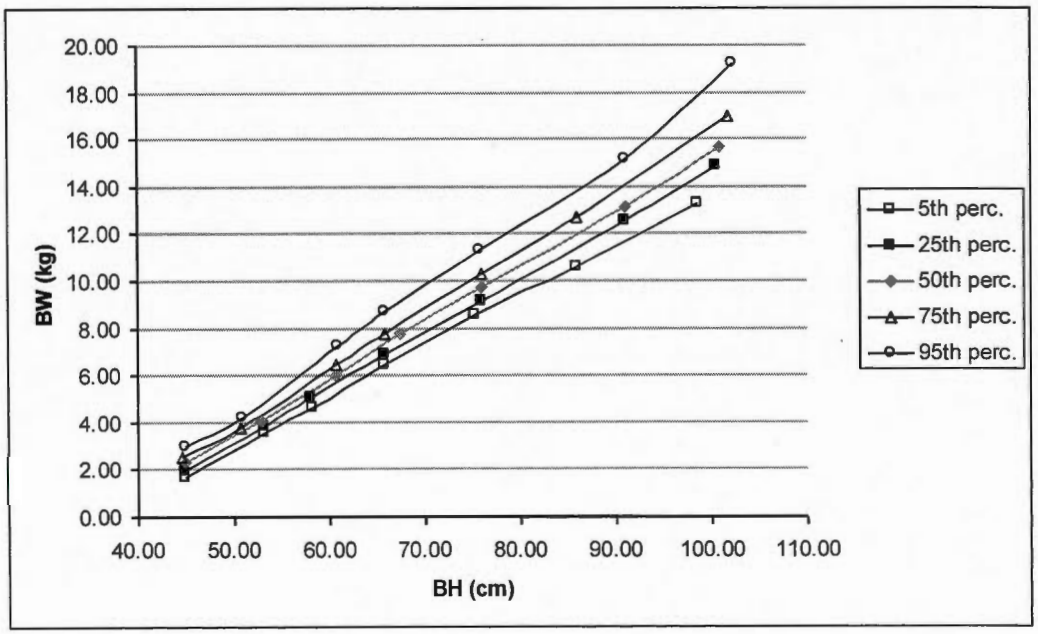

Figure 1.S4. Body weight vs body height chart for females under 3 years of age. Symbols represent extracted data (from CDC, 2007) used for interpolation in the PBPK model.

Impact of adipose tissue:blood partioning and metabolism on toxicokinetic profile

Sensitive parameters that are difficult to obtain from epidemiological study questionnaires are Clint and those involved in the adipose tissues to blood partition coefficient (Flfa, Flfc, and Flb). To compare impact of variability on lifetime toxicokinetic profiles in the sensitive parameters Clint and those involved in the adipose tissues to blood partition coefficient (Flfa, Flfc, and Flb) with that obtained in section 3.1, MC simulations were performed by varying the parameters according to distributions published in the literature (Table 1.S4). The results are shown in Figures 1.S6 and 1.S7. Using average values of these parameters can lead to errors representing 1.5 or 1.8 times the true values in blood concentrations for Clint or lipid composition in adipose tissues and blood, respectively.

Monte-Carlo simulations using distributions for BH, BW and all sensitive parameters

Using available distribution data for sensitive parameters a Monte-Carlo simulation was made to determine the expected variability in blood concentration following a given background daily dose (20 ng/kg/day). Variability in exposure to breast milk was also included and milk concentration was set at 2 μg/L. Results, shown in Figure 1.S8, show that regardless of external exposure levels, internal concentrations can vary greatly in a population simply due to variability in physiology, breastmilk drinking in childhood, and more importantly breastfeeding during adulthood (20 to 40 years of age). Blood concentrations vary up to 100-fold during adulthood years when lactation occurs.

Table 1.S4. Distribution of sensitive parameters.

Parameter	Type of distribution	Mean value	Standard deviation	Minimum value	Maximum value	Reference
Intrinsic clearance factor	Log normal	1	0.5	-	-	Assumed[1]
Fraction of lipids in blood	normal	0.006	0.0014	-	-	Estimated from Patterson et al 1988**
Fraction of lipids in fat (adult)	uniform	-	-	0.71	0.87	Pelekis et al 2003
Fraction of lipids in fat (child)	uniform	-	-	0.53	0.65	Pelekis et al 2003
Milk drinking duration	uniform	1	-	0	2	Assumed
Fraction of lipids in milk	normal	3.8	0.8	-	-	Neville et al. 1991
Duration of lactation	uniform	-	-	0	2	Assumed
Volume of milk factor	normal	1	0.05	-	-	Neville et al. 1991

*Variability of intrinsic clearance assumed to vary in similar proportions as in Nong and Krishnan (2006).

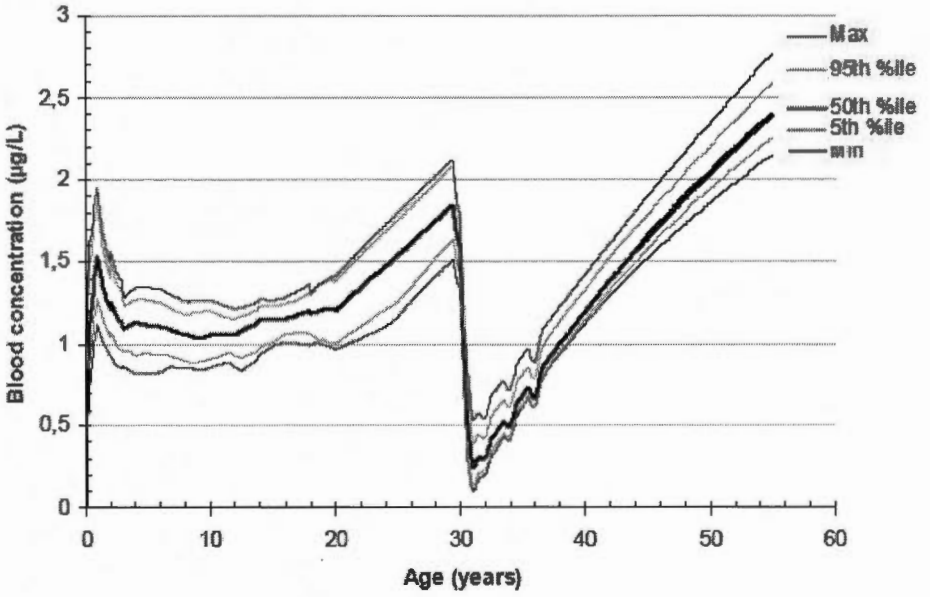

Figure 1.S5. Monte Carlo simulations representing the impact of body height and body weight variations within a population on PCB153 lifetime toxicokinetics. Lines represent simulations of blood concentrations for various body weights and body heights: minimum, 5th percentile, 50th percentile, 95th percentile and maximum values. Other parameters were kept the same for all simulations.

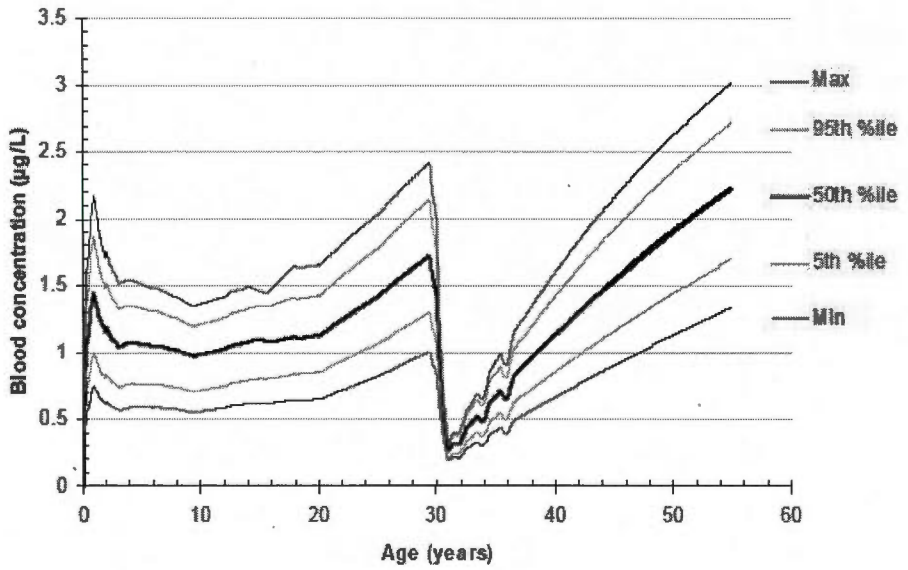

Figure 1.S6. Monte Carlo simulations representing the impact of variations in lipid composition of adipose tissue and blood within a population (see Table 1.S4) on PCB153 lifetime toxicokinetics. Lines represent simulations of blood concentrations for various body weights and body heights: minimum, 5th percentile, 50th percentile, 95th percentile and maximum values. Other parameters were kept the same for all simulations. Body weight and height profiles were those of the 50th percentile. All other parameters were the same for all simulations.

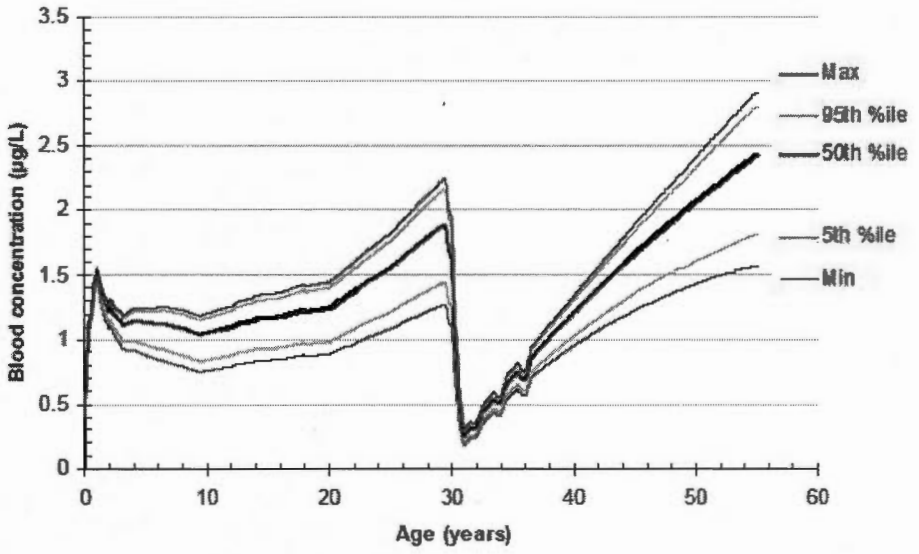

Figure 1.S7. Monte Carlo simulations representing the impact of variations in intrinsic clearance (see Table 1.S4) within a population on PCB-153 lifetime toxicokinetics. Lines represent simulations of blood concentrations for various body weights and body heights: minimum, 5th percentile, 50th percentile, 95th percentile and maximum values. Other parameters were kept the same for all simulations. Body weight and height profiles were those of the 50th percentile. All other parameters were the same for all simulations.

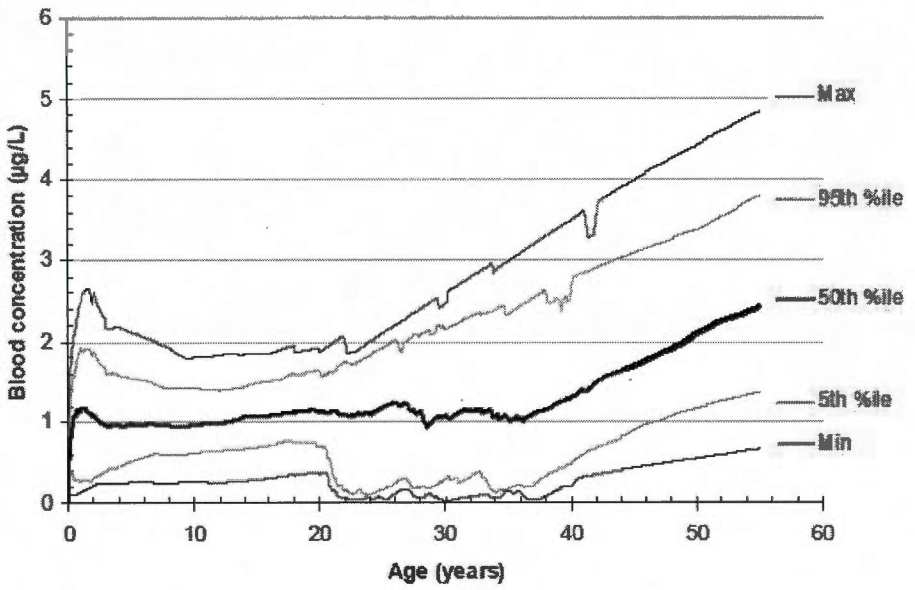

Figure 1.S8. Monte Carlo simulations representing the impact of variations in all sensitive parameters, including body height and weight, within a population (see Table 1.S4) on PCB153 lifetime toxicokinetics. Lines represent simulations of blood concentrations for various body weights and body heights: minimum, 5th percentile, 50th percentile, 95th percentile and maximum values. Other parameters were kept the same for all simulations. All other parameters were the same for all simulations.

Chapitre II

A CASE STUDY ADRESSING THE RELIABILITY OF POLYCHLORINATED BIPHENYL (PCB) LEVELS MEASURED AT THE TIME OF BREAST CANCER DIAGNOSIS IN REPRESENTING EARLY LIFE EXPOSURE

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Abstract

Background: To date, breast cancer epidemiologic studies have relied upon blood or tissue specimens sampled at the time of diagnosis or a few years prior to assess lifetime exposure to PCBs. In this study, we evaluated whether such PCB measurements are indicative of early life levels by reconstructing lifetime toxicokinetic profiles for women included in the CECILE case-control study using a physiologically based pharmacokinetic (PBPK) model.
Methods: We simulated lifetime toxicokinetic profiles of PCB-153 for 2134 French women by incorporating information on body weight history, height, pregnancies and breast-feeding in the PBPK model. Oral dose was calculated by considering measured blood PCB-153 and the temporal trend of environmental contamination. Area under the concentration versus time curve (AUC) for each decade of life and maximum blood concentration (Cmax) were compiled and compared to measured levels using Pearson partial correlation analyses adjusting for age at diagnosis.
Results: When considering all individuals, simulated AUCs correlated with measured PCBs with coefficients ranging from 0.735 to 0.981. The weakest correlations were obtained with AUCs for the first decades of life. Stratified analyses suggested that breast-feeding reduces the reliability of late life blood levels in representing lifetime exposure.
Conclusion: Results of this study suggest that PCB levels measured at the time of diagnosis do not fully represent early life exposures.
Impact: PBPK-derived estimates of early life levels circumvent the limitations of current approaches in assessing PCB lifetime exposure and may be used to address hypothesized windows of breast vulnerability (e.g. puberty) in this population.

Key words: Breast cancer, polychlorinated biphenyls (PCBs), physiologically-based pharmacokinetic modeling (PBPK), exposure assessment, case-control study.

2.1 Introduction

A large number of epidemiologic studies and reviews on polychlorinated biphenyls (PCBs) exposure and breast cancer have been published over the last 20 years. Overall, available evidence does not support an association between exposure to PCBs and breast cancer incidence (Negri *et al.*, 2003; Golden et Kimbrough, 2009). Most of these studies considered blood PCB levels monitored at the time of diagnosis or a few years prior to be proxies for cumulated lifetime exposure. Given the inter-individual variations in environmental and physiological parameters affecting PCB toxicokinetics, it is questionable whether these levels provide accurate information on the internal exposure of women during their earlier decades of life.

Several epidemiologic studies raised concerns in regards to organochlorine exposure assessment. When interpreting the results obtained with exposure estimation based on single biologic samples, many researchers cautioned readers that although levels at the time of study might be indicative of past exposure, it remains uncertain to which extent these levels allow an appropriate evaluation of PCB carcinogenic potential during hypothesized early life periods of vulnerability (Brody et Rudel, 2003; Laden *et al.*, 2001; Raaschou-Nielsen *et al.*, 2005; Wolff *et al.*, 2000). A prospective study published by Cohn *et al.* (2007) supported such contention as blood 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (DDT) measured around 26 years of age was associated with increased odds of developing breast cancer while most studies based on DDE levels (DDT metabolite) at later stages reported no association (Lopez-Cervantes *et al.*, 2004). Thus, when taking into account the theory of breast vulnerability during certain time-windows, one might question the value of PCB levels measured in samples taken around the time of cancer diagnosis to address exposure-disease associations.

To overcome the exposure assessment issue, we have previously developed a physiologically based pharmacokinetic (PBPK) model that allows the estimation of lifetime PCB internal levels while taking into account individual information on physiology and reproductive

history (Verner *et al.*, 2008). In the present study, we aimed to assess the appropriateness of blood PCB levels measured at the time of diagnosis in representing earlier exposures by comparing these values to PBPK-derived lifetime toxicokinetic profiles of French women included in a population based case-control study.

2.2 Methods

2.2.1 Population

Data from a total of 2135 French women included in the CECILE population-based case-control study who accepted to give blood for organochlorine quantification was used. Cases were women diagnosed with invasive or *in situ* breast cancer between February 2005 and June 2007 within the administrative regions of Ille-et-Vilaine and Côte d'Or in France. Controls were matched to breast cancer cases by 5-year age groups through random-digit dialing procedure in the same residence area. One individual with no information on weight was excluded.

2.2.2 PCB quantification

PCB-153 was selected as a proxy for a mixture of PCB congeners because it was detected in blood samples more than twice as frequently than the other congeners and its levels were correlated to other individual congeners and to the sum of 3 congeners (PCB-138, -153 and -180). Samples were prepared by liquid-liquid extraction followed by a solid phase extraction. PCB-153 concentration in samples was determined by gas chromatography coupled to an ion trap mass spectrometer detector. The limit of detection (LOD) for PCB-153 was 0.50 µg/L. When levels were below the LOD (n=898, 42 % of study participants), values were randomly generated based on the log-normal distribution function as well as woman's age and body mass index (BMI) change over the 10 last years, two determinants of PCB-153 levels at the time of diagnosis in this cohort (Bachelet *et al.*, 2009). Whole blood PCB-153 concentration was adjusted on a lipid basis according to the equation in Akins *et al.*(1989).

2.2.3 Physiologically-based pharmacokinetic (PBPK) modeling

PBPK modeling is a pharmacokinetic tool that describes the physiological, biochemical and physicochemical processes governing the absorption, distribution, metabolism and excretion of a xenobiotic, thus enabling the simulation of its kinetics blood and tissue. Many of these

processes are dependent upon several physiological parameters such as the volume and composition of organs, as well as the blood perfusion. The mathematical functions describing these processes are derived from population data and are designed to allow the incorporation of different profiles of body weight and height. In the case of organochlorine compounds like PCBs, it is paramount to take women reproductive history into account as these chemicals are extensively excreted through breast-feeding. In this study, we simulated individualized lifetime toxicokinetic profiles with a previously published PBPK model (Verner *et al.*, 2008) modified as detailed in Verner *et al.* (2009). This framework integrates women weight profile (reported for each decade of life – missing weights were imputed through multiple linear regression [n=212]), height, age at deliveries, duration of each breast-feeding period and date of birth. Consumption of breast milk in infancy was not considered given the dearth of information on maternal levels and duration of breast-feeding. Considering the wide variation in reported PCB-153 half-life values (Shirai et Kissel, 1996), simulations were carried out with half-lives of 10 and 30 years. Simulations were performed using the software acslX (Aegis Technologies Group, Inc., Huntsville, AL, USA). Examples of simulated toxicokinetic profiles are depicted in Figure 2.1 A and B.

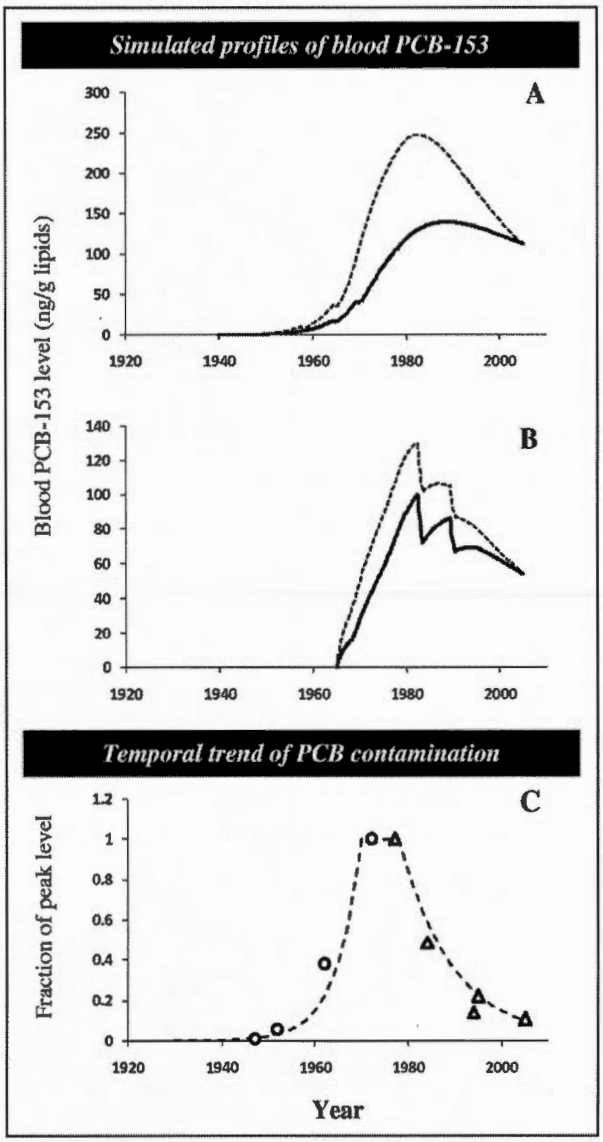

Figure 2.1. Examples of toxicokinetic profiles for women born in 1940 (A) and 1965 (B) with different weight and breast-feeding profiles. Lifetime blood PCB-153 profiles for half-lives of 10 (dotted line) and 30 years (continuous line) were based on levels at diagnosis, individual characteristics and the temporal trend in actual environmental contamination levels (C) reported las fractions of peak levels (based on production data (○) (OCDE, 1982) and estimated daily intakes (Δ) (Baars *et al.*, 2004)).

2.2.4 Environmental exposure estimation

The daily oral dose estimation included an assessment of temporal trends in environmental levels. Because PCB production started in the 1930s, we considered environmental exposure to be null before that date. Exposure from 1930 until 1970 was characterized using production data as no daily intake estimations were available for Europe until that time (OCDE, 1982). Maximum exposure was assumed during the 1970s when peak production occurred and peak daily intake estimations were made. We defined the decline in PCB daily intake after 1977 with European data taken from Baars *et al.* (2004). These temporal data were transformed into fractions of peak values reached in the 1970s (*F*, ranging from 0 to 1). The trend in *F* values is depicted in Figure 2.1 C. To integrate this temporal trend in the PBPK model, we allowed the oral dose input to change over time based on the following equation:

$$Dose = F \times Maximum\ daily\ dose \qquad [1]$$

The maximum daily dose (in the 1970s) was optimized for each woman by iterative simulations to obtain a toxicokinetic profile with matching simulated and measured blood PCB-153 concentration at the time of sampling.

2.2.5 Statistical analyses

The area under the lipid-adjusted blood concentration versus time curve (AUC) for each decade, a proxy for total internal exposure during a time period, as well as the maximum blood concentration (Cmax) were compiled for each of the 2134 women (see Figure 2.1). AUC for the first decade (0-10 years) was not used in this study as levels during this period are strongly influenced by breast milk consumption in infancy (Barr *et al.*, 2006), a factor that was not included in our simulations. PBPK-derived exposure estimates were compared to measured blood PCB-153 concentrations through Pearson partial correlation analyses adjusting for age at cancer diagnosis stratified by 5 year intervals to account for case-control matching procedures in this study. We assessed these correlations in different age, breast-

feeding and BMI strata. Statistical analyses were performed using SPSS for Windows 10.0 (SPSS Inc., Chicago, IL, USA).

2.3 Results

Women enrolled in this study were on average 55 years old and ranged from 25 to 75 years (Table 2.1). Breast-feeding was relatively low in this population as 47 % of the women never breast-fed, and those who breast-fed did so for an average period of 5.6 months over their lifetime. Only 6 % of the women breast-fed for longer than 12 months. Mean BMI was 24.8 and 13 % of individuals had a BMI over 30. Measured blood PCB-153 levels ranged from < LOD to 1218 ng/g lipids.

2.3.1 Blood PCB-153 levels

Median simulated PCB-153 Cmax (5^{th}-95^{th} percentile) were 244 (68 - 703) and 126 (38 - 353) ng/g lipids for half-lives of 10 and 30 years, respectively. The Cmax was reached on average 24 and 18 years prior to blood sampling when assuming half-lives of 10 and 30 years. Cmax values were on average 2.8 times higher than blood PCB-153 levels measured at the time of diagnosis when simulations were performed with a 10 year half-life, and 1.6 times higher when a half-life of 30 years was assumed.

Table 2.1. Demographic characteristics of study participants.

	Categories	N	%	Mean	SD	Minimum	25th p.	Median	75th p.
	Maximum								
Age (years)		2134		55.0	10.85	25 .5	47.3	55.3	63.4
	75.0								
	< 40	210	10						
	40-50	489	23						
	50-60	695	33						
	> 60	740	34						
Parity	0	191	9						
	1	292	14						
	2	802	38						
	3+	849	40						
Total breast-feeding		1005	47	5.6	7.43	0.2	1.4	3.0	7.0
	64.7								
(months)	0	1129	53						
	0-3	444	21						
	3-12	437	20						
	12+	124	6						
BMI (kg/m^2)		2131		24.8	4.8	14.3	21.3	23.8	27.2
	54.7								
	< 20	252	12						
	20-25	1045	49						
	25-30	565	26						
	> 30	272	13						
Height (cm)		2131		161.5	6.2	134	157	161	165
	.180								
Blood PCB-153 (ng/g lipids)*		2134		84.9	86.7	4.2	56.2	88.3	142.5
	1218.3								

* After imputation of PCB-153 levels below the LOD

2.3.2 Correlation analyses

Exposure estimates and blood levels were log-transformed prior to analyses. Because correlation coefficients between measured PCB-153 and AUCs displayed a similar pattern regardless of the half-life used in the analyses, only results obtained with a half-life of 30 years are reported. AUCs during contiguous decades were correlated with coefficients of 0.900 between AUC 10-20 and AUC 20-30, 0.666 between AUC 20-30 and AUC 30-40, 0.724 between AUC 30-40 and AUC 40-50, and 0.959 between AUC 40-50 and AUC 50-60. When all the individuals were included in the analyses, Pearson partial correlations between measured blood PCB-153 levels at diagnosis and simulated estimates decreased from 0.981 for AUC 50-60 (closest to sampling time) to 0.735 for AUC 10-20 (furthest from sampling time) (Table 2.2). Stratification by age groups did not reveal any apparent discrepancy across correlation coefficients. However, the higher coefficients in these strata when compared to those obtained with the whole dataset suggested that a residual effect of age was not accounted for by matching women by 5 year intervals. Stratification based on the duration of breast-feeding revealed that the loss in correlation strength across time is, at least in part, due to breast-feeding, with women who breast-fed for longer than 1 year showing the weakest correlations between measured levels and AUCs before the reproductive period ($r=0.509$ for AUC 10-20 years). Stratification on BMI did not impact the trend in correlation coefficients. Running correlation analyses solely for women with no missing weight data did not change the results. When only women with blood levels above the LOD were included in the analyses, correlations were on general slightly weaker but the impact of breast-feeding on correlation coefficients was diminished. This could be the result of the shorter duration of breast-feeding in this subgroup.

Table 2.2. Pearson partial correlation coefficients between PCB-153 levels measured at the time of cancer diagnosis and PBPK-derived exposure estimates (adjusted by age at blood sampling).

	PBPK-derived estimates of exposure					
	Cmax	AUC10-20	AUC20-30	AUC30-40	AUC40-50	AUC50-60
Age at diagnosis						
< 40	0.903	0.891	0.902			
40-50	0.937	0.883	0.933	0.972		
50-60	0.965	0.824	0.919	0.960	0.978	
> 60	0.972	0.847	0.857	0.912	0.963	0.981
Total breast-feeding (months)						
0	0.971	0.751	0.744	0.867	0.961	0.982
< 3	0.976	0.752	0.751	0.884	0.969	0.981
3-12	0.932	0.743	0.751	0.892	0.967	0.984
> 12	0.837	0.509	0.564	0.786	0.948	0.960
BMI (kg/m^2)						
< 20	0.965	0.770	0.799	0.917	0.981	0.989
20-25	0.948	0.715	0.726	0.880	0.967	0.981
25-30	0.959	0.775	0.747	0.848	0.960	0.985
> 30	0.943	0.747	0.751	0.873	0.955	0.973
All	0.951	0.735	0.736	0.870	0.963	0.981

2.4 Discussion

We conducted this study to address the reliability of PCB exposure levels measured at the time of breast cancer diagnosis in representing early life exposure. Results suggested that certain parameters can influence PCB toxicokinetics and hinder the reliability of levels sampled at the time of cancer diagnosis in representing exposure during early life. Among these parameters, the variability in the cumulative duration of breast-feeding was shown to weaken the correspondence between blood PCB measurements and estimated early-life levels, even with the low prevalence of breast-feeding in this population. The traditional approach to adjust for breast-feeding in exposure-disease associations is unlikely to prevent exposure misclassification as it does not account for the timing of breast-feeding periods and their impact on blood/tissue PCB levels during periods which could be etiologically relevant in breast cancer development. This also applies for variations in BMI. Therefore, samples collected at the time of diagnosis or a few years before may not allow the identification of associations between breast cancer and PCB levels during earlier hypothesized periods of susceptibility such as puberty.

The theory of critical windows of breast vulnerability was put forward in studies of atomic bomb survivors in Japan. These studies showed that women below 20 years of age at the time of exposure to radiations were more likely to develop breast cancer than women exposed during later stages of life (Wakeford, 2004). Cohn *et al.* (2007) also suggested that early chemical insults, as indicated by DDT levels measured during the twenties, can increase breast cancer incidence. If there is a critical period of susceptibility to PCBs during early stages of life such as puberty, results reported herein suggest that studies based on blood levels at the time of diagnosis may not be able to detect exposure-disease associations. The observed discrepancy between levels measured in this study and estimated past levels during hypothesized critical windows could have biased odds ratios (ORs) towards the null hypothesis in epidemiologic studies. Assessment of exposure during etiologically relevant periods through pharmacokinetic modeling is expected to decrease exposure misclassification, as compared to the simple use of blood PCB levels measured many years after the period of breast vulnerability to carcinogens. Under the assumption of a real link

between PCBs and breast cancer, PBPK modeling should thus reinforce the observed association (i.e., the OR) between PCB exposure estimate and disease. Preliminary analyses conducted in the CECILE study point to this direction (manuscript in preparation).

Because the reported correlation analyses were based on PBPK model simulations rather than serial blood PCB-153 measurements, some limitations of this study ought to be mentioned. First, many factors such as changes in dietary habits or place of residence were not included in the model and could have altered the extent of environmental exposure. Also, the PBPK model as a whole was not validated on repeated PCB measurements. Be that as it may, this approach incorporating breast-feeding history and weight/height profiles allows the reconstruction of lifetime PCB levels considering validated population-derived physiological parameters relevant to PCB toxicokinetics. Given the scarcity of studies with repeated PCB measurements, the PBPK model presented herein offers a unique opportunity to evaluate the adequacy of PCB levels measured at the time of cancer diagnosis to study exposure-disease associations.

In conclusion, this study suggests that epidemiologic studies based on PCB levels measured at the time of diagnosis or a few years before might have underestimated associations that are specific to early life periods of vulnerability. These results imply that future studies on PCBs and other lipophilic persistent organic pollutants will need to further evaluate the temporal variability in internal levels, especially when studying diseases with early windows of susceptibility. While prospective studies on diseases with long latency periods are methodologically challenging, serial blood/tissue sampling would help understand the impact of different physiologic and lifestyle factors on the toxicokinetics of these compounds. Based on these data, statistical or pharmacokinetic models could be developed and calibrated to facilitate the back-estimation of levels during different periods of exposure.

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CHAPITRE III

A PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODEL FOR THE ASSESSMENT OF INFANT EXPOSURE TO PERSISTENT ORGANIC POLLUTANTS IN EPIDEMIOLOGIC STUDIES

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Abstract

Background: It has been suggested that pre- and postnatal exposure to persistent organic pollutants (POPs) can promote several adverse effects in children, such as altered neurodevelopment. Epidemiologic studies to date have relied on the analysis of biological samples drawn pre- or postnatally for exposure assessment, an approach that might not capture some key events in the toxicokinetics of POPs.

Objectives: We aimed to build a generic physiologically-based pharmacokinetic (PBPK) modeling framework for neutral POPs to assess infant toxicokinetic profiles and to validate the model using data on POP levels measured in mothers and infants from a Northern Quebec Inuit population.

Methods: The PBPK model developed herein was based upon a previously published model to which an infant sub-model was added. Using the model and maternal blood levels at the time of delivery, exposure to 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE), 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT), hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), 2,2',3,4,4',5-hexachlorobiphenyl (PCB-138), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) and 2,2',3,4,4',5,5'-hexachlorobiphenyl (PCB-180) in mothers was estimated to subsequently simulate infant blood, breast milk, and cord blood POP concentration. Simulations were then compared with corresponding measured levels through Spearman correlation analyses.

Results: Predictions were highly correlated with measured concentrations for PCB-153, PCB-180, PCB-138, HCB, and *p,p'*-DDE (r = 0.83 to 0.96). Weaker correlations were observed for *p,p'*-DDT and β-HCH for which levels were near the limits of detection.

Conclusion: This is the first study to validate a PBPK model of POPs in infants on an individual basis. This approach will reduce sampling efforts and enable the use of individualized POP toxicokinetic profiles in the epidemiologic studies of POP adverse effects on child development.

Keywords: epidemiology, exposure assessment, infants, persistent organic pollutants, physiologically-based pharmacokinetic modeling.

3.1 Introduction

Many epidemiologic studies suggest that pre- and postnatal exposure to persistent organic pollutants (POPs) can promote several adverse effects in children, such as altered neurodevelopment (Eskenazi *et al.*, 2008; Koopman-Esseboom *et al.*, 1996;Ribas-Fito *et al.*, 2001; Rosas et Eskenazi, 2008; Walkowiak *et al.*, 2001). Although environmental levels of most POPs declined after their use was restricted in many countries, these compounds are still found in most human tissues and blood samples. Moreover, some persistent chemicals, such as polybrominated diphenyl ethers (PBDEs) and 1,1,1-trichloro-2,2-bis(*p*-chlorophenyl)ethane (*p,p'*-DDT), are still produced and widely used.

Prenatal and early postnatal periods are critical for the developing brain. Mild endocrine disruption during these time windows can lead to deficits in neuropsychological functions in infants, and these effects are specific to the time when hormonal dysregulation occurs (Zoeller et Rovet, 2004). Therefore, timing of exposure to endocrine-disrupting compounds during early stages of development is critical. Epidemiologic studies to date have relied on diverse biological samples drawn prenatally, at birth or postnatally to investigate relationships between exposure to POPs and various outcomes pertaining to health and development. Although multiple samples provide information on the overall exposure in infants, this approach is costly, time-consuming, and subject to ethical limitations and might not capture some key events in the lifetime toxicokinetic profiles of POPs. Therefore, new exposure assessment tools to estimate complete toxicokinetic profiles in infants might broaden the scope of findings for specific periods of susceptibility.

Several studies have attempted to describe POP toxicokinetics in humans through various modeling approaches Ayotte *et al.* (2003) developed a statistical model to predict levels of PCB-153 in Inuit infants. Their multivariate model that included maternal PCB-153 plasma lipid concentration, breast-feeding duration, and the sum of two skin-fold thicknesses (an index of infant adipose tissue mass) explained 72 % of PCB-153 infant plasma concentration variance at 6 months postpartum. However, predictions were limited to a single compound at a specific age and such data-based models might not be applicable to other ranges of

exposure and populations because of genetic and life habit differences. Single-compartment first-order pharmacokinetic models were used by Lorber and Phillips (2002) and LaKind *et al.* (2000) to assess exposure to dioxin-like compounds through breastfeeding. Although these models generated toxicokinetic profiles in infants, they integrated infant body burden at birth and breast milk concentration as independent variables, an approach that would require the sampling of both media within an epidemiologic study context. Furthermore, such models do not allow estimations of POP levels in target tissues.

Physiologically-based pharmacokinetic (PBPK) modeling enables the estimation of tissue and blood POP levels based on physiologic parameters, such as organ volume and blood flow, as well as compound physico-chemical properties. PBPK models have been developed to evaluate infant exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) through breastfeeding (Ayotte *et al.*, 1996; Gentry *et al.*, 2003; Kreuzer *et al.*, 1997). Although this is a relevant approach to assess POP tissue dosimetry in humans, PBPK frameworks to date have not allowed the integration of individual physiologic characteristics (e.g. body mass index lifetime profiles), thus preventing their use within the context of epidemiologic studies. Moreover, no attempts were made to validate these models on a large scale using longitudinal POP measurements, and on multiple persistent chemicals. To generate individualized toxicokinetic profiles to be used within epidemiologic studies, we recently developed a PBPK framework to assess lifetime internal exposure to different POPs in women that is based on the physiology and reproductive history of the subjects (Verner *et al.*, 2008). Based on this framework, the model presented herein was developed to generate individualized toxicokinetic profiles in infants exposed pre- and postnatally.

In this study, we aimed to develop a generic model to estimate infant exposure to POPs through placental transfer and breastfeeding, and to validate the model using data on levels of three polychlorinated biphenyls (PCB) congeners [2,2',3,4,4',5-hexachlorobiphenyl (PCB-138), 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) and 2,2',3,4,4',5,5'-hexachlorobiphenyl (PCB-180)], hexachlorobenzene (HCB), β-hexachlorocyclohexane (β-HCH), 1,1-dichloro-2,2-bis(*p*-chlorophenyl)ethylene (*p,p'*-DDE) and *p,p'*-DDT measured in mothers and infants

in the course of an infant cohort study in the Inuit population of Nunavik (Northern Quebec, Canada).

3.2 Methods

The development of the PBPK model followed a four-step approach: model representation, parameterization, simulation and validation. The PBPK model presented herein was based on a previous model (Verner *et al.*, 2008) modified to consider additional information on events related to pregnancy, lactation, and infant physiology.

3.2.1 Model Representation

The model representation for the mother was described previously (Verner *et al.*, 2008). Briefly, the maternal model was functionally described as a tissue network of nine compartments (liver, brain, adipose tissue, richly perfused tissues, poorly perfused tissues, mammary tissue, uterus, placenta, and fetus) (Figure 3.1). POPs were assumed to be fully absorbed through ingestion of contaminated food and were described as a direct input into the liver compartment. Excretion through lactation was represented as an output from the mammary tissue compartment.

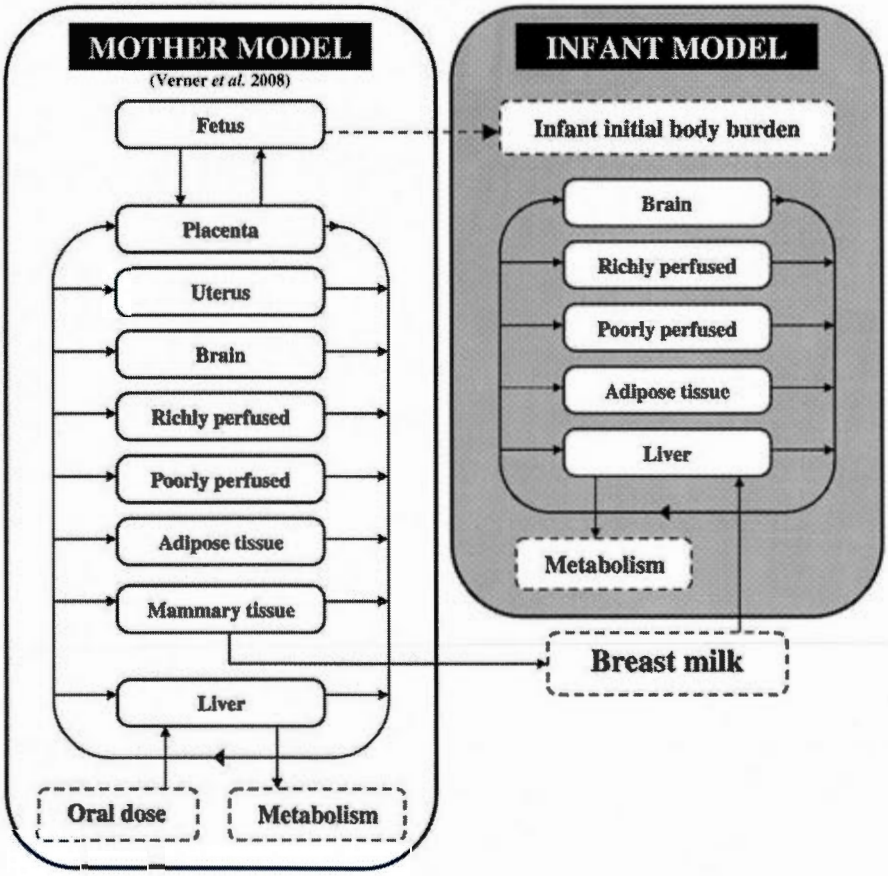

Figure 3.1. Conceptual representation of the mother-infant PBPK model. A previously published model for the mother (left) was modified to integrate an infant sub-model (right). Initial infant body burden was calculated as detailed in the methods section.

To describe the exposure/toxicokinetics in infants, we integrated a 5 compartment sub-model (i.e., liver, adipose tissue, richly perfused tissue, poorly perfused tissue, brain) with the maternal model (Figure 3.1). Infant liver was defined as the first-pass organ where both POP intake from breastfeeding and POP metabolism take place. Adipose tissue represented the main storage compartment for POPs. Richly and poorly perfused tissues compartments represented groups of organs that were lumped according to their perfusion rates and residence times. Finally, the brain was represented as a specific compartment because it is the target organ in relation to potential neurodevelopment deficits. The female infant model was conceptually different from the maternal model, because compartments relating to pregnancy and lactation (i.e., uterine, placental, and mammary tissues) were kinetically irrelevant for the first year of life.

Absorption

As previously mentioned, we described oral absorption of POPs for both mothers and infants as a direct input in the liver compartment. The assumption of 100 % absorption is supported by high POP absorbed fractions reported by McLachlan (1993) and Maruyama *et al.* (2003). We assumed dietary exposure to POPs in mothers to be a constant daily dose adjusted for body weight. Infant postnatal exposure to POPs was limited to the ingestion of contaminated breast milk up to 12 months of age. Because of the lack of information on mixed feeding (partial breastfeeding), only the exclusive breastfeeding period was considered. Infant intake was calculated as follows:

$$\text{Intake} = \text{CMilk} \times \text{Qmilk} \qquad [1]$$

where Intake is the hourly intake through breastfeeding in micrograms per hour, CMilk is the POP concentration in milk (μg/L), and Qmilk is the milk intake in liters per hour.

Infant initial body burden was based on the assumption that intrauterine exposure to POPs is blood flow limited. Because lipophilic POPs distribution is solely driven by their solubility in

lipids, infant blood and tissue lipid-adjusted concentrations at birth were equal to lipid-adjusted levels in maternal blood at the time of delivery.

Distribution

The distribution of POPs in compartments was managed by both the blood flow and tissue:blood partition coefficients. This process was described by a set of mass balance differential equations (MBDE) that assume homogenous distributions in tissues, as follows:

$$\frac{dAt}{dt} = Qt \times \left(Ca - \frac{Ct}{P_{t:b}} \right) \qquad [2]$$

where At represents the amount of chemical in the compartment (micrograms), Qt is the blood flow perfusing the compartment (liters per hour), $P_{t:b}$ is the tissue:blood partition coefficient for the compartment, and Ca and Ct stand for concentrations in arterial blood and the tissue (micrograms per liter), respectively. Venous and arterial blood concentrations were assumed to be equal, the latter being calculated as a weighed sum of tissues venous blood concentration:

$$Ca = \sum \frac{Qt \times Cvt}{Qc} \qquad [3]$$

where Ca is the arterial blood concentration (micrograms per liter), Qt is the blood flow to tissues (liters per hour), Cvt is the tissue venous blood concentration (micrograms per liter), and Qc is the cardiac output (liters per hour).

Metabolism

Metabolism was limited to the liver compartment and the rate was described by the product of the hepatic extraction ratio (Eh), the liver blood flow in L/hr (Ql) and the arterial blood concentration in micrograms per liter (Ca) entering the compartment, as follows:

$$RAM = Eh \times Ql \times Ca \qquad [4]$$

The hepatic extraction ratio was calculated as a function of liver volume adjusted intrinsic clearance in liters per hour per kilogram liver (*Cl*int), blood flow to liver in liters per hour (Ql), and liver volume in liters (Vl):

$$Eh = \frac{Cl\,\text{int} \times Vl}{Cl\,\text{int} \times Vl + Ql} \qquad [5]$$

Intrinsic clearance values were assumed to be equal in mothers and infants. Ontogenic changes in enzymes catalyzing POP biotransformation were not included in this study but could easily be implemented in the future.

Excretion

Excretion of POPs through breastfeeding was detailed in our previous study (Verner *et al.*, 2008). Briefly, POP excretion through breast milk was managed by milk flow out of the mammary tissue compartment and milk:blood partition coefficient. Placental transfer of POPs was modeled as an initial body burden in infants where tissue and blood lipid-adjusted concentrations were equal to maternal plasma lipid-adjusted POP concentration.

3.2.2 Model Parameterization

Maternal parameters

Maternal parameters were those of the previously published model (Verner *et al.*, 2008), with modifications for blood lipids during pregnancy as well as breast milk lipids and daily excreted volume. Blood lipid profile throughout pregnancy and postpartum period was modeled as a 70% linear increase in neutral lipids from the start of pregnancy until delivery, and a linear decrease back to normal values over 1.5 months postpartum. This profile was derived from data on triglycerides and cholesterol throughout and after pregnancy (Chiang *et al.*, 1995).

Breast milk volume and lipid content were modified from our previously published article. A better description of ingested milk volume as a function of both infant age and infant body weight was found in Arcus-Arth *et al.* (2005) and was modified as follows:

$$\text{Intake} = -0.114 \times \text{Age}_i + 0.158 \qquad [6]$$

where Age_i is the infant age in years and daily milk intake is in liters per kilogram of infant body weight. According to this equation, the breast milk daily intake is assumed to be 0.158 L/kg of infant body weight at birth and to decline to 0.044 L/kg of infant body weight at the age of 12 months. Breast milk lipid composition was also modified: a logarithmic function was optimized on breast milk lipid fraction data from Bitman *et al.* (1983) for the first 42 days and Arcus-Arth *et al.* (2005) for the period 3-12 months:

$$\text{FlMilk} = 0.0034 \times \ln(\text{Age}_i) + 0.0414 \qquad [7]$$

where FlMilk represents the fraction of lipids in milk, and Age_i is the infant age in years. Given this equation, the fraction of lipids in breast milk at the time of delivery is set to approximately 0.0101 and rapidly increases to reach 0.0414 one year postpartum.

Infant parameters

Equations and parameters describing infant physiology were allowed to change as a function of sex, age, body weight and body height. Age- and sex-specific organ volumes and blood flows are presented in Table 3.1. Tissue:blood and milk:blood partition coefficients were calculated with the approach of Haddad *et al.* (2000) which showed that the distribution of chemicals with log octanol:water partition coefficient (Kow) values > 4 is driven solely by lipid fractions in tissues and blood:

$$P_{t/m:b} = \frac{Fl_{t/m}}{Fl_b} \qquad [8]$$

where $P_{t/m:b}$ is the tissue:blood or milk:blood partition coefficient, $Fl_{t/m}$ stands for the fraction of lipids in tissue or milk, and Fl_b represents the fraction of lipids in blood. Fraction of lipids in tissues and blood are shown in Table 3.2. Values for richly and poorly perfused tissues were calculated from volume-adjusted lipid fractions of organs they represent. Equations and

Table 3.1 Physiologic parameters for male and female infants from birth to 12 months of age

Parameters	Sex	Equations
BS[a]	M & F	$BW^{0.515} \times (BH^{0.422}) \times 234.9$
Volumes (L)		
Liver (Vl)[a]	M & F	$0.05012 \times BW^{0.780}$
Adipose tissue (Vat)[a]	M & F	$0.91 \times BW - (Vl + Vrp + Vpp + Vsk)$
Skin tissue (Vsk)[a]	M & F	$0.664 \times (BS/10^4) + 0.07850 \times (BS/10^4)^{1.048}$
Richly perfused tissue (Vrp)[a]	M	$-0.01068 \times AGE_i + 2.038 \times (BW^2/BH)^{0.4014} - 0.2046 - Vl$
	F	$-0.01919 \times AGE_i + 3.193 \times (BW^2/BH)^{0.2657} - 1.374 - Vl$
Poorly perfused tissue (Vpp)[a]	M & F	$Vt + Vh + Vsm$
Tongue (Vt)[a]	M & F	$0.00119 \times BW - 0.0004302$
Heart (Vh)[a]	M	$0.0000001017 \times [(BH^{0.6640}) \times (BW^{0.3951}) \times 242.7]^{1.420}$
	F	$0.0000001017 \times [(BH^{0.6962}) \times (BW^{0.3561}) \times 242.7]^{1.420}$
Skeletal muscle (Vsm)[a]	M	$0.09561 \times BW + 0.01601 \times BH + 0.1097 \times AGE_i$
	F	$0.09563 \times BW + 0.01650 \times BH + 0.09102 \times AGE_i - 0.1642$
Brain (Vbrain)[b]	M	$10 \times (AGE_i + 0.213)/(6.030 + 6.895 \times AGE_i)$
	F	$10 \times (AGE_i + 0.226)/(6.521 + 7.514 \times AGE_i)$
Blood flows (L/hr)		
Cardiac output (Qc)[a]	M	$0.2519 \times BW^{0.7609} \times 60$
	F	$0.2508 \times BW^{0.7815} \times 60$
Liver (Ql)[a]	M	$0.84 \times Vl \times 60$
	F	$Vl \times 60$
Adipose tissue (Qat)[a]	M	$0.0209 \times Vat \times 60$
	F	$0.0300 \times Vat \times 60$
Richly perfused (Qrp)[a]	M & F	$Qc - (Qpp + Qat + Ql + Qbrain)$
Poorly perfused (Qpp)[a]	M	$(0.03 \times (Vt + Vsm) + 0.73 \times Vh) \times 60$
	F	$(0.03 \times (Vt + Vsm) + 0.96 \times Vh) \times 60$
Brain (Qbrain)[c]	M & F	$-0.0024 \times AGE_i^4 + 0.1305 \times AGE_i^3 - 2.4822 \times AGE_i^2 + 18.025 \times AGE_i + 15.197$

values were taken from Price K *et al.* (2003a). Because no significant variation was seen in these compartments' lipid fractions during the first year of life, these values were kept constant.

Metabolism was parameterized from half-life values as described previously (Verner *et al.*, 2008). Briefly, half-life values were transformed into liver volume-adjusted intrinsic clearance values (Table 3.3) in order to calculate hepatic extraction ratios.

3.2.3 Model Simulation and Validation

Variability assessment

To grasp the possible range of under- and overprediction we may expect due to interindividual variability in sensitive physiologic parameters [sensitivity analysis shown in the Supplemental Material (online at http://www.ehponline.org/members/2008/080047/suppl.pdf], we performed Monte Carlo analyses on three independent sensitive parameters relevant to POP toxicokinetics for infants exposed through breastfeeding (100 iterations). In this

Table 3.2 Fraction of lipids in infant tissues

Tissue	Age (years)	Fraction of lipids
Blood[a]	0–1	0.005
Adipose tissue[a]	0	0.347
	0.5	0.472
	9	0.550
Liver[a]	0	0.021
	1	0.041
Richly perfused[b]	0–1	0.018
Poorly perfused[b]	0–1	0.021
Brain[a]	0	0.026
	1.5	0.061

Table 3.3 Half-life and calculated liver volume-adjusted intrinsic clearnace of POPs in infants and mothers

Compounds	Half-life (years)	Intrinsic clearance (L/hr/kg liver)	Source of half-life value
PCB-153	27.5	0.0082601	Yakushiji et al. (1984)
PCB-180	9.9	0.0229504	Yakushiji et al. (1984)
PCB-138	16.3	0.0144328	Yakushiji et al. (1984)
HCB	6.0	0.0395820	To-Figueras et al. (1997)
β-HCH	7.6	0.0309631	Jung et al. (1997)
p,p'-DDE	15.0	0.0156840	Wolff et al. (2000)
p,p'-DDT[a]	5.0	0.0144328	Smith (1999)

analysis, published SD values for the fraction of lipids in infant adipose tissue (0.12), the fraction of lipids in breast milk (0.0032), and the daily ingested breast milk consumption (0.038 L/kg infant body weight/day) were used to randomly assign parameter values in each iteration, assuming normal distributions (Arcus-Arth *et al.*, 2005; Bitman *et al.*, 1983; White *et al.*, 1991).

We used body weight and height profiles for the 50th percentile taken from the Center for Disease Control and Prevention (CDC) data as surrogate physiologic profiles for both mother and infant. The analysis was done for a woman giving birth to a girl at the arbitrary age of 25, followed by a 3-month breastfeeding period. Mothers were exposed to 10 ng/kg body weight/day of the highest and lowest half-life compounds used in this study (PCB-153 and *p,p'*-DDT).

Validation dataset

Validation of the model was done using data on PCB-180, PCB-153, PCB-138, HCB, β-HCH, *p,p'*-DDE, and *p,p'*-DDT levels in Inuit mothers and infants from northern Quebec (Canada). The cohort was described previously by Muckle *et al.* (2001). The subjects kept for the validation step were those for which sufficient information was available:

- Mother physiology (pre-pregnancy weight, height, age at delivery)
- History of lactation (exclusive breastfeeding period)
- Infant physiology (body weight and body height information for a period covering the time of infant plasma sampling)
- Dates of sampling (mother and infant plasma)

- POP concentrations in mothers and infants at 6 months of age (with levels above the limit of detection).

Simulations

To run lifetime exposure in each mother, the PBPK model required complete body weight and body height profiles. Because maternal body height and weight were available only at one time before pregnancy, we used growth curves to infer lifetime profiles. This was done by assigning a percentile body height or body height profile from CDC data to fit the individual pre-pregnancy body weight and height. Maternal exposure was optimized to fit the lipid-adjusted POP level in plasma. Infant body weight and height were linearly interpolated between measurements taken at 0, 6, and 12 months. Simulated lipid-adjusted infant plasma (at 6 months of age), breast milk and cord blood levels were then compared with measured levels through correlation analyses.

Software

We performed PBPK modeling and simulations using ACSLXtreme software (Aegis Technologies Group, Inc., Huntsville, AL, USA). We performed non-parametric correlation analyses (Spearman coefficients) using SPSS for Windows statistical package (SPSS Inc., Chicago, IL, USA).

3.3 Results

3.3.1 Monte Carlo simulations

We assessed population variability for given physiologic profiles and breastfeeding history using Monte Carlo simulations based on variability of three independent sensitive parameters (breast milk consumption, fraction of lipids in breast milk, and fraction of lipids in infant adipose tissue). Simulations for PCB-153 and *p,p'*-DDT yielded distributions of toxicokinetic profiles in infants during their first 6 months (Figure 3.2). At the end of the breastfeeding period (3 months postpartum), the 5^{th}–95^{th} percentile values in infant blood concentration were 227-538 μg/kg lipids for PCB153 and 90-213 μg/kg lipids for *p,p'*-DDT. The 5^{th}–95^{th} percentile ranges at 6 months of age were 119-303 and 42-109 μg/kg lipids for PCB153 and *p,p'*-DDT, respectively. These ranges indicated a ~ 2.5-fold variability between the 5^{th} and 95^{th} percentiles.

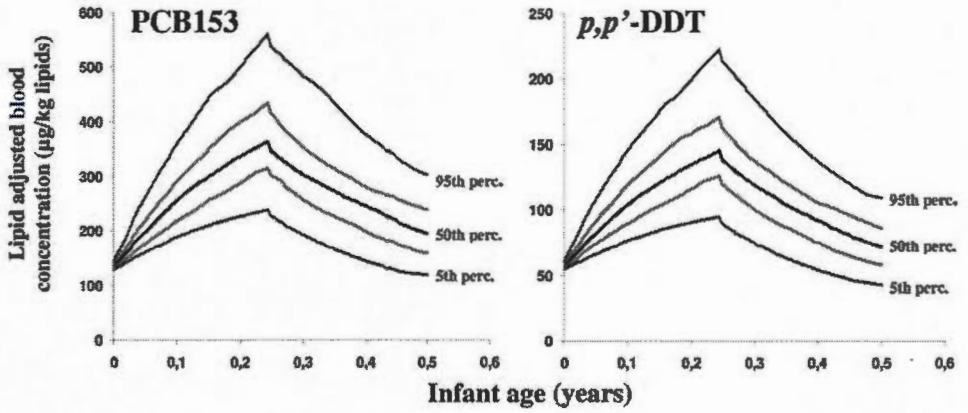

Figure 3.2. Blood POP level distributions in infants obtained from Monte Carlo simulations. Simulations were carried out by varying three independent sensitive parameters (100 iterations). Black lines represent the 5^{th}, 50^{th} and 95^{th} percentiles whereas grey lines represent the 25^{th} and 75^{th} percentiles. Toxicokinetic profiles were simulated for a mother exposed to a constant dose of 10 ng/kg/day, and giving birth to a girl at the age of 25 followed by a 3-month breastfeeding period. Simulations were performed for the highest and lowest half-life compounds in this study, PCB153 and *p,p'*-DDT.

3.3.2 Simulation of maternal POP toxicokinetics

To simulate the lifetime toxicokinetics of POPs in mothers, exposure was optimized to fit plasma concentration in samples drawn around the time of delivery. Lowest and highest exposure values used to match maternal levels were kept for the determination of environmental exposure ranges. Estimated maternal exposure ranges were (ng/kg body weight/day): 1.3-52.3 for PCB-153, 1.5-31.8 for PCB-180, 0.9-34.9 for PCB-138, 1.6-58.9 for HCB, 0.2-4.4 for β-HCH, 5.2-212.5 for *p,p'*-DDE and 0.4-25.7 for *p,p'*-DDT.

3.3.3 Prediction of cord blood concentration

As mentioned above, cord blood lipid-adjusted concentration was assumed to be the same as the maternal lipid-adjusted blood concentration at the time of delivery. Using this assumption, strong Spearman's correlations ($r > 0.90$) were obtained for most POPs when comparing simulated values with measured values as depicted in Figure 3.3. Weaker correlations were observed for *p,p'*-DDT ($r = 0.79$) and β-HCH ($r = 0.35$). No systematic under- or overestimation was observed for cord blood levels prediction. Comparison could not be achieved for HCB, as this chemical was not analyzed in cord blood.

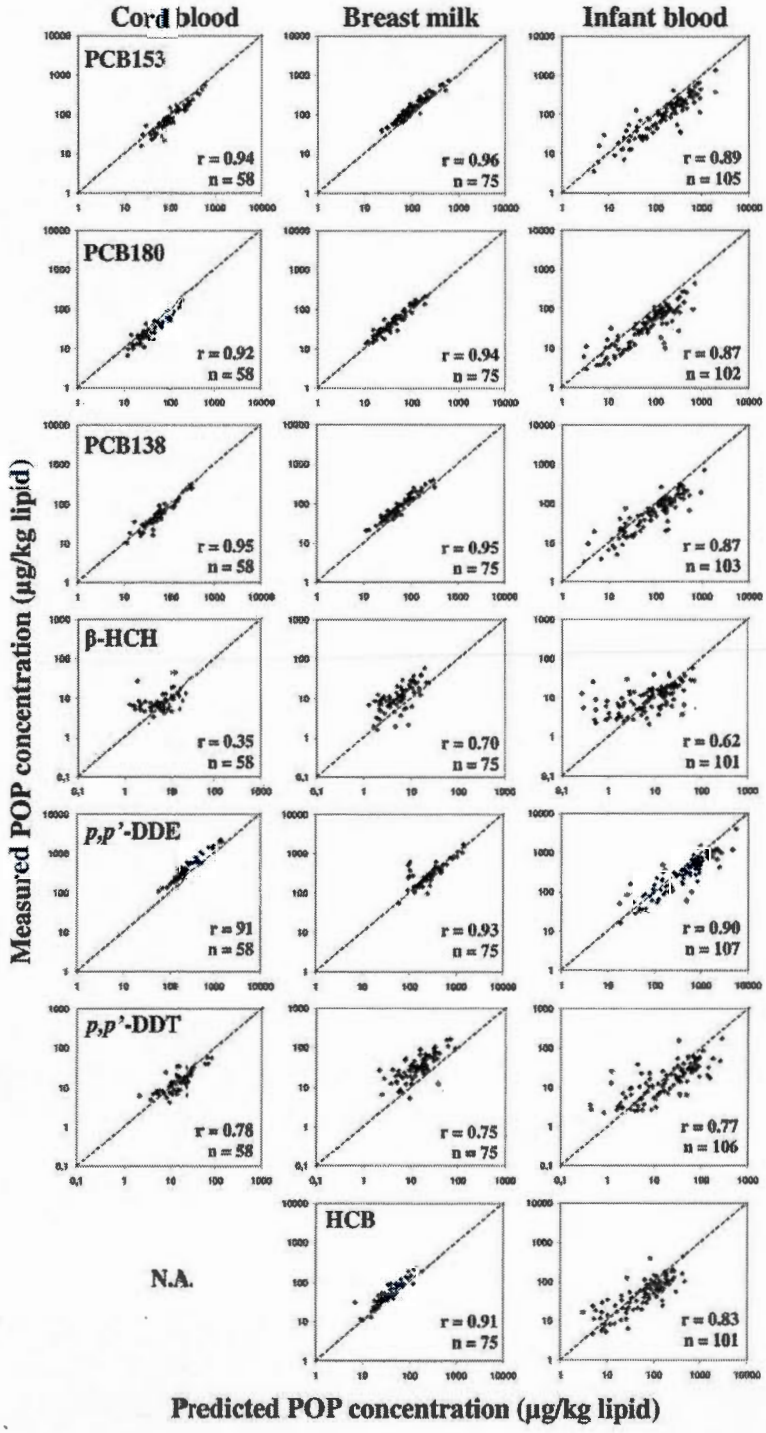

Figure 3.3. Spearman's correlations between predicted and measured lipid adjusted POP levels in infant plasma, cord blood and breast milk. Dotted lines represent the unity slope. Correlation analysis for HCB levels in cord blood could not be conducted since this compound was not measured in this media.

3.3.4 Prediction of breast milk concentration

Because breastfeeding was considered to be the exclusive route of postnatal exposure in infants for this study, simulated breast milk concentrations were compared with levels in samples collected approximately 1 month after delivery. Most correlations had Spearman's rho values above 0.90 (Figure 3.3). *p,p'*-DDT and β-HCH simulated breast milk levels had weaker correlation values of 0.72 and 0.70, respectively. A slight tendency to underestimate breast milk concentrations was observed for most compounds.

3.3.5 Estimation of infant blood concentration at ~ 6 months of age

Infant blood levels were predicted considering both exposure through placental transfer and breastfeeding. Simulated toxicokinetic profiles matched measured infant blood POP concentration with high Spearman's correlations (Figure 3.3). Plots of measured values on predicted values showed a slight systematic overestimation (Figure 3.3). Simulations of β-HCH toxicokinetics in infants yielded inconsistent results, displaying a high variability that could not be explained by the model at low concentrations. Predicted blood concentrations fell within the 2.5-fold range variation defined in Monte Carlo simulations (between the 5th and 95th percentiles) for 88% (*p,p'*-DDE), 85 % (PCB-153 and PCB-138), 82% (HCB), 75 % (PCB-180), 67 % (*p,p'*-DDT) and 66 % (β-HCH) of individuals.

3.4 Discussion

In this study we aimed to build and validate a PBPK model for the characterization of infant exposure to POPs through placental transfer and breastfeeding. This work follows our first modeling paper on the assessment of lifetime POP toxicokinetics in human (Verner *et al.*, 2008) and focuses primarily on exposure in infants. The successful validation of our PBPK model within this study using data on mothers and infants from an Inuit population further supports the potential of this internal exposure assessment tool in epidemiologic studies.

This PBPK model based on individual physiology and breastfeeding period allows the integration of several concurrent physiological events (e.g. pre- and postpartum changes in maternal physiology, lactation, infant growth) that are relevant to POP toxicokinetics. Modeling simultaneous variations in volume and lipid composition of maternal tissues and breast milk over time is critical in characterizing POP distribution and thus infant exposure. The determination of infant initial body burden is also essential to correctly assess perinatal exposure, because initial levels rapidly depurate due to infant growth. The toxicokinetic profiles depicted in Figure 3.4 show the close relationship between POP levels in maternal blood, breast milk and infant blood. Another benefit of using PBPK modeling is the possibility of predicting POP concentration in potential target tissues of newborns and infants. However, such estimates were not shown in the present study, as their validation would have required the sampling of infant tissues.

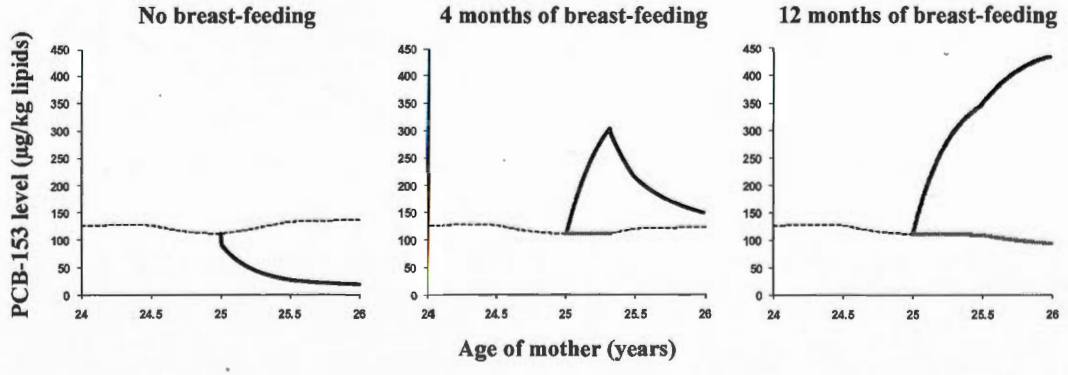

Figure 3.4. Examples of toxicokinetic profiles for PCB153 in maternal blood (dotted line), breast milk (grey line) and infant blood (black line) for different breastfeeding scenarios. Breast milk and maternal blood concentrations overlap across the breast-feeding period.

To estimate background exposure to POPs in mothers, the daily intake was optimized so the simulated lipid-adjusted blood concentration reached the measured maternal blood level. A study by Dewailly *et al.* (1996a) estimated the mean daily PCB exposure in Inuits to be 13.8 µg/day, i.e., a 230 ng/kg body weight/day intake for an average 60-kg woman. In their review, van Oostdam *et al.* (2005) reported median daily intakes of 20 nanograms per kilogram body weight per day for HCB, 40 ng/kg body weight/day for *p,p'*-DDT and 50 ng/kg bw/day for PCBs in Inuits of Qikiqtarjuaq. The estimated daily exposure ranges (ng/kg body weight/day) obtained in this study for *p,p'*-DDT (0.4-25.7), HCB (1.6-58.9), and the sum of the three PCBs (3.7-119) were comparable to these daily intake estimates, indicating that the PBPK model is fairly accurate at estimating the maternal exposure to POPs. As reported previously (Verner *et al.*, 2008), daily intakes can be better assessed when considering pregnancies and lactation periods that precede the time of sampling, as well as dietary habits and temporal trends in environmental levels. When available, such information could easily be integrated in the PBPK model.

Predicted cord blood, breast milk and infant blood concentrations by PBPK modeling showed strong Spearman's correlations with measured levels (see Figure 3.3). However, a minor discrepancy still remains between simulated and measured levels, indicating that sources of toxicokinetic variability are not accounted for within the model. Monte Carlo simulations showed that the infant toxicokinetic profile is influenced by the variability in breast milk lipids and volume, as well as the lipid fraction in the adipose tissue compartment. Variability within these sensitive parameters is not taken into account when simulating POP toxicokinetics, leading to potential errors in predictions. Simulated values fell within the 2.5-fold variability range for 66-88 % of individuals used in this study, depending on the compound. This suggests that other factors such as inaccuracy in information from questionnaires and variability in the analytic methods used to quantify POPs might affect model predictability. It is also possible that some parameters were incorrectly estimated as the physiologic equations were derived from data on Caucasians, whereas the data set used in this study was on Inuit people. The under- or overestimation of infant adipose tissue volume

might yield a systematic bias and other measurements to estimate this value, such as the sum of skinfolds (Ayotte *et al.*, 2003), should be evaluated in future studies.

Another source of error can arise from the fact that exposure to POPs through the ingestion of contaminated breast milk was limited to the exclusive breastfeeding period. Because of a lack of information, the period of mixed feeding (when the infant is fed both breast milk and formula) was assumed to be a period without breastfeeding, leading to a potential underestimation of infant exposure in cases with important mixed feeding. A detailed description of formula and milk consumption history would provide valuable information for the estimation of exposure through breastfeeding during the mixed feeding period and allow the inclusion of partial breastfeeding in the model.

Correlations between predicted and measured levels were weaker for β-HCH and *p,p'*-DDT. These compounds are characterized by their shorter half-lives and levels in mothers and infants that are near the limits of detection. The impact of half-life alone on model inability to precisely estimate measured values is not likely to be the most influent factor, given that simulated levels of HCB (a compound with a shorter half-life than β-HCH) were strongly correlated to measured concentrations in infant plasma and breast milk. On the other hand, model accuracy might be limited when working with POP levels close to limits of detection, a phenomenon potentially caused by reduced precision in analytic methods at low POP concentration in samples. Predictions with β-HCH might have been influenced by the fact that this compound has a lower log Kow (3.81). Its value below the partitioning cut-off (i.e., log Kow = 4) for the method used in this study possibly led to a slight error in partition coefficients determination. Overall, caution should be exerted when using this model for compounds with levels near the limits of detection and/or that have low log Kow values.

Simulations slightly underestimated POP lipid-adjusted concentration in breast milk. This might be explained by the difference in approaches to adjust POP levels for lipids. Adjustment for blood lipids in the Ayotte *et al.* (2003) study was based on total lipids as described in Phillips *et al.* (1989), whereas the PBPK model used neutral lipid equivalents exclusively. Lipids in breast milk are composed almost solely of triacylglycerols (Jensen,

1999), whereas lipids in maternal blood also contain significant levels of cholesterol and phospholipids (Berghaus *et al.*, 1998). Although triacylglycerols and cholesterol are neutral lipids in which POP will be stored, phospholipids have a lipo-hydrophilicity similar to a mixture of 30% neutral lipids and 70% water (Poulin et Krishnan, 1995). Therefore, including phospholipids in blood lipid content calculation leads to an artifactual difference between blood and milk lipid-adjusted POP concentrations. As only neutral lipid equivalents are considered in the PBPK model and POPs are assumed to be homogeneously distributed in lipids, simulated lipid-adjusted POP levels in blood and milk are equal. Thus, the underestimation of POP levels in breast milk can be explained at least partially by these different approaches in adjusting levels for lipid contents. When information on whole blood and breast milk lipid composition is available, levels should be adjusted on neutral lipid equivalents rather than total lipids.

The results of this study showed the potential of PBPK modeling in estimating POP toxicokinetics in infants. Detailed information on internal exposure, such as the timing and amplitude of the maximum concentration, can be harvested from the simulations (Figure 3.5). This could be an important input in epidemiology to study high exposure during hypothesized critical time windows. For example, it was suggested that internal exposure to PCB alters thyroid hormones levels (Chevrier *et al.*, 2007). Disturbed thyroid levels in neonates can result in several adverse health effects such as visual, motor, language, and memory impairment (Zoeller et Rovet, 2004). Using only measured POP levels in 6-month old infants to test an exposure-effect hypothesis might be too limiting for the complete analysis of time- and dose-related responses. An approach using the maximum concentration (and the time when this concentration is reached) as well as the area under the curve for different time frames might provide crucial information on critical windows of exposure as well as dose-response relationships.

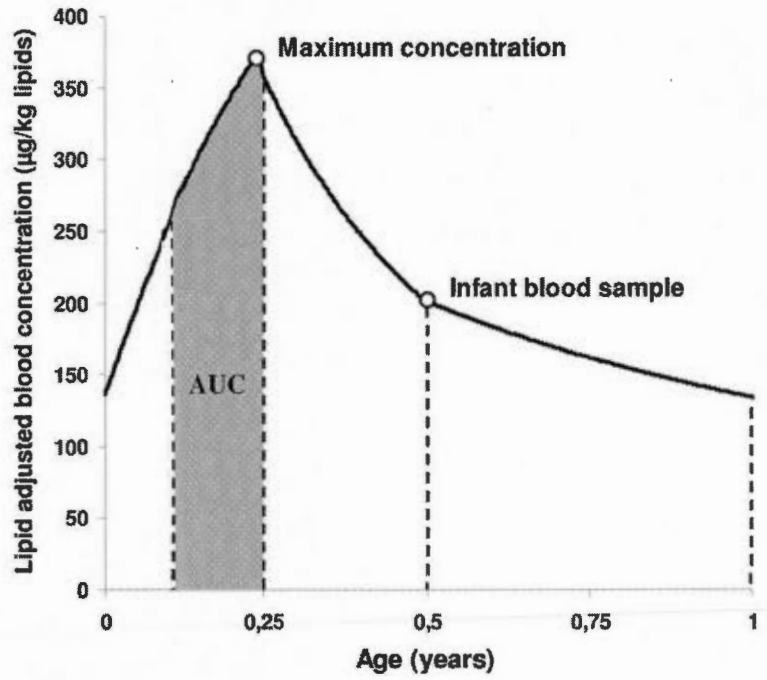

Figure 3.5. Graphic representation of information to be harvested from simulations. The maximum concentration and the area under the curve (AUC) represented by the area shaded in grey are examples of information to be extracted from the toxicokinetic curve.

Overall, PBPK modeling was shown to be a relevant method to assess pre- and postnatal exposure to POPs. The presented model allows the prediction of infant exposure through placental transfer (cord blood level estimation at the time of delivery) and breastfeeding strictly from information on maternal blood levels and physiologic profiles that can be easily gathered from epidemiologic questionnaires. Moreover, this is the first study to validate a PBPK model of POPs in infants on an individual basis. This study also successfully demonstrates how our previously published model (Verner *et al.*, 2008) adequately describes the lactational excretion of POPs in women. This PBPK modeling approach will permit the assessment of exposure to POPs in infants prospectively and retrospectively, therefore reducing sampling efforts and enabling the use of individualized POP toxicokinetic profiles in epidemiologic studies. Further research is planned to validate the model with other populations, later life stages, and additional compounds such as PBDEs. Researchers interested in collaborating with us or using our model are encouraged to contact us.

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Supplemental material

Sensitivity analysis

The sensitivity analysis was carried out by assessing the impact of a 10% increase in parameter values on lipid adjusted blood concentration of PCB153 or area under the curve (AUC) in infants for the first year of life. The scenario of a 25 year old woman giving birth to an infant and breastfeeding for 6 months was used for this exercise. Figure 3.S1 is a graphical representation of the sensitivity analysis that we performed.

Results show that independent parameters FLFi, Vmilk and FLmilk have a strong influence on lipid adjusted blood concentration and AUC in infant. The figure also presents sensitivity for dependant parameters VFi, VSPi and VRPi. POPs being highly distributed mainly in the adipose tissue compartment, it is not surprising that the volume of this compartment (VFi) has an effect on blood levels. On the other hand, VSPi and VRPi appear to be sensitive but their impact can be explained by the fact that they are used in the calculation of VFi. VFi was not included in the Monte Carlo analysis because of the inter-dependence between this parameter and many others (changing the volume of it would affect other parameters).

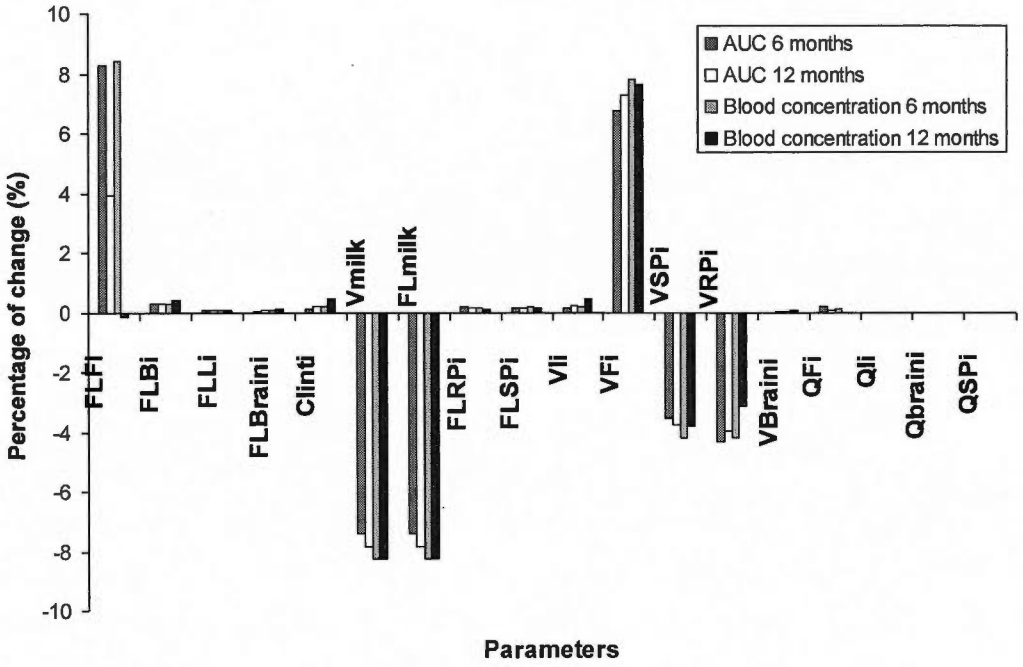

Figure 3.S1. Sensitivity analysis for infant parameters. The impact of a 10% increase in parameter value on blood PCB153 level is displayed as a percentage of change in the AUC for the first 6 months (dark grey bars) and 12 months of life (white bars), as well as blood PCB153 concentration at 6 months (light grey bars) and 12 months (black bars). Parameter abbreviations: FLFi = fraction of lipids in adipose tissue, FLBi = fraction of lipids in blood, FLLi = fraction of lipids in liver, FLBraini = fraction of lipids in brain, FLBi = fraction of lipids in blood, FLRPi = fraction of lipids in richly perfused tissues, FLSPi = fraction of lipids in slowly (poorly) perfused tissues, Vmilk = volume of breast milk ingested by day, FLmilk = fraction of lipids in breast milk, Vli = volume of liver, Vfi = volume of adipose tissue, VSPi = volume of slowly (poorly) perfused tissues, VRPi = volume of richly perfused tissues, VBraini = volume of brain.

CHAPITRE IV

ALTERATION OF INFANT ATTENTION AND ACTIVITY BY POLYCHLORINATED BIPHENYLS: UNRAVELLING CRITICAL WINDOWS OF SUSCEPTIBILITY USING PHYSIOLOGICALLY-BASED PHARMACOKINETIC MODELING

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Abstract

Pre- and postnatal exposure to polychlorinated biphenyls (PCBs) can impair behavioural function in animal models at doses within the range at which humans are commonly exposed. Yet, epidemiologic studies conducted in the US and Europe are inconsistent with regard to the developmental effects of lactational exposure to these chemicals. This inconsistency may be due to limitations in the current methodological approaches for assessing postnatal exposure to PCBs. Our study used a physiologically-based pharmacokinetic (PBPK) model to simulate blood PCB levels during specific pre- and postnatal periods and to evaluate the relation of those levels to infant behaviour. A previously validated PBPK model was used to simulate infant blood PCB-153 levels at delivery and on a month-by-month basis during the first year of life for Inuit infants enrolled in a longitudinal birth cohort. Infant behaviour was assessed using the Behaviour Rating Scales (BRS) of the Bayley Scales of Infant Development (BSID-II) at 11 months of age and video coding of inattention and activity measured during the administration of the mental development subscale of the BSID-II. The estimated pre- and postnatal PCB exposure measures predicted significant increases in inattention and activity at 11 months. Whereas inattention was related to prenatal exposure, activity level, measured by non-elicited activity, was best predicted by postnatal exposure, with the strongest association obtained for simulated PCB levels during the 4th month of life. These findings are consistent with previous reports indicating PCB-induced behavioural alteration in attention and activity level. Simulated infant toxicokinetic profiles for the first year of life revealed windows of susceptibility during which PCBs may impair infant attention and activity.

Key words: Polychlorinated biphenyls, behaviour, infants, physiologically-based pharmacokinetic modeling, prenatal, postnatal

4.1 Introduction

Infants are exposed to substantial quantities of environmental contaminants through breast-feeding. Among the multitude of compounds detected in human milk, polychlorinated biphenyls (PCBs) are found at measurable levels worldwide although their production and use were banned in the 1970s. Experimental studies on nonhuman primates revealed long-lasting behavioural impairment following a 20-week postnatal dosing regimen that led to blood concentrations within the range of human exposure (Rice, 1999). Given the experimental evidence of PCB-induced developmental deficits, there are concerns that breast milk contamination by PCBs might trigger adverse health outcomes in infants and perhaps counteract the beneficial effects of breast-feeding.

Multiple studies have demonstrated that low-level prenatal exposure to PCBs can impair neurodevelopment in infancy and childhood (Boucher *et al.*, 2009; Jacobson *et al.*, 1985; Ribas-Fito *et al.*, 2001; Rogan *et al.*, 1986; Schantz *et al.*, 2003; Stewart *et al.*, 2000). Although the absolute quantities of PCBs transmitted to the infant via lactation exceed the quantities transmitted prenatally across the placenta by several fold (Jacobson *et al.*, 1984), only a few studies have linked postnatal PCB exposure to adverse effects on development (Jorissen, 2007). While results from two European studies suggest that mother-infant transfer of PCBs through breast-feeding is associated with poorer developmental outcome—lower scores on the Bayley Scales of Infant Development (BSID-II) in one study (Koopman-Esseboom *et al.*, 1996), on the Kaufman Assessment Battery for Children in the other (Walkowiak *et al.*, 2001)—, US-based studies have found no associations between postnatal exposure through lactation and scores on the Fagan Test of Infant Intelligence, Mullen Scales of Early Learning, MacArthur-Bates Communicative Development Indices, and BSID during infancy, or on Wechsler Intelligence Scales for Children IQ (WISC-III) and academic achievement tests at school age (Gladen *et al.*, 1988; Jacobson et Jacobson, 1996; Jacobson *et al.*, 1985; Pan *et al.*, 2009). The failure to detect adverse effects from breast-feeding may be due to the limitations in the current methodological approaches for assessing postnatal exposure to PCBs.

To date, studies have relied on samples of breast milk and/or infant blood collected at a single time point to characterize postnatal exposure. Because infant toxicokinetics are the result of complex interactions among several concurrent events, including breast-feeding and growth, simple exposure metrics based on measurement on a single occasion provide only partial insight into early-life toxicokinetic profiles. To overcome the limitations of traditional exposure assessment, we recently developed and validated a physiologically-based pharmacokinetic (PBPK) model to simulate toxicokinetic profiles for infants exposed to persistent organic pollutants pre- and postnatally (Verner *et al.*, 2009). Using maternal blood PCB level, duration of breast-feeding, and weight/height profiles for mothers and infants, this model displayed high predictability in estimating PCB concentrations in cord blood, breast milk and infant blood during the first year of life. Simulated toxicokinetic profiles allow the consideration of PCB levels during different time windows, an approach that may enable epidemiologic studies to identify critical periods of susceptibility to PCB neurotoxicity.

This study aimed to examine time-specific associations between simulated PCB levels and indicators of infant behavioural function in an Inuit population from Nunavik (Canada). We used 2,2',4,4',5,5'-hexachlorobiphenyl (PCB-153) as a surrogate for the environmental mixture of PCBs found in biological specimens of the Inuit since this compound has a long biologic half-life and was shown to be highly correlated to other frequently detected PCB congeners in plasma and breast milk in this cohort (Ayotte *et al.*, 2003; Muckle *et al.*, 2001).

4.2 Materials and methods

4.2.1 Study design and population

Sample characteristics are given in Table 4.1. A subset of this sample is further detailed in Muckle *et al.* (2001). Briefly, 333 Inuit women from three villages (Puvirnituk, Inukjuaq and Kuujuaraapik) located on the Hudson Bay coast in Arctic Quebec were enrolled in a prospective longitudinal study at their first or second prenatal medical examination between 1995 and 2002. Exclusionary criteria were participation in the study with a previous child (13.5 %), loss to follow-up (2.4 %), refusal to participate (20.3 %), neonatal death (3.0 %), failure to obtain biological sample (3.6 %), and relocation to another village (3.9 %). A maternal blood sample was drawn at delivery or within a few weeks postpartum. Cord blood was collected for those who gave birth at the community hospital or nursing station. A sample of infant blood was taken at the age of ~ 6 months for a subset of the cohort. PCBs, mercury, lead, other chlorinated compounds and lipids were analyzed in blood samples at the Centre de Toxicologie du Québec with the methodologies detailed by Muckle *et al.* (2001). Fatty acids were measured in plasma phospholipids at the University of Guelph Lipid Analytical Laboratory as described by Jacobson *et al.* (2008). Infant growth and neurodevelopment were assessed at 6 and 11 months of age. The study sample consisted of the 168 infants for whom PCB-153 level was available from cord or maternal blood, information on duration of breast-feeding was provided by the mother, and the 11-month neurodevelopmental assessment was completed.

Table 4.1. Characteristics of participants

	No	%	Mean ± SD	Range
Maternal characteristics				
Pre-pregnancy weight (kg)	168		61.9 ± 11.6	42.2 - 110.0
Age at delivery (years)	168		25.0 ± 5.9	14.6 – 40.9
Parity	168		2.0 ± 1.8	0 - 9
Blood PCB-153 (ng/g lipids)	164		134.0 ± 112.3	14.6 - 709.0
Milk PCB-153 (ng/g lipids)	124		170.0 ± 138.6	25.4 - 842.5
Smoking during pregnancy				
Yes	162	96		
No	6	4		
Number of cigarettes/day	162		10.7 ± 6.0	1 - 27.5
Drinking during pregnancy				
Yes	97	58		
No	71	42		
Number of drinks/day [a]	97		0.10 ± 0.25	0.00 - 1.76
Child-rearing environment				
Primary caregiver's socioeconomic status	168		16.7 ± 10.9	5 - 40
Years of education	168		8.9 ± 1.7	5.5 - 14.3
Married / living with partner				
Yes	116	69		
No	52	31		
Infant characteristics				
Gender				
Female	70	42		
Male	98	58		
Birth weight (g)	168		3468 ± 577	1620 - 4870
Age at 11 month assessment (days)	167		349.5 ± 37.8	291 - 503
Adoption status				
Adopted	20	12		
Not adopted	148	88		
Premature (< 37 weeks)				
Yes	39	23		
No	129	77		
Duration of exclusive breast-feeding (days)	168		155.9 ± 133.1	0 - 466
Cord blood measures				
PCB-153 (ng/g lipids)	85		112.3 ± 96.6	15.7 - 550.9
p,p'-DDE (ng/g lipids)	85		376.3 ± 305.0	55.7 - 1773.4
Lead (µmol/L)	86		0.23 ± 0.17	0.03 - 0.86
Mercury (µmol/L)	84		107.9 ± 79.7	12.0 - 485.0
DHA/AA	83		0.41 ± 0.15	0.12 - 1.08
Behavioural indicators				
Non-elicited activity				
% of examination time	150		20.4 ± 8.4	2.9 - 54.3
Rate of events/min	150		3.7 ± 1.1	0.8 - 6.8

Inattention			
% of examination time	153	12.2 ± 5.4	0.0 - 26.4
Rate of events/min	153	3.4 ± 1.4	0.0 - 7.3
BRSb orientation/engagement	168	42.0 ± 6.1	15 - 53
BRS emotional regulation	168	33.7 ± 4.6	20 - 47
BRS motor quality	167	31.4 ± 3.4	22 - 40
BRS total	167	113.5 ± 9.6	74 - 136

[a] One standard drink of alcohol corresponds to 0.5 oz of absolute alcohol (AA), which is the equivalent of 12 oz or 350 ml of beer, 6 oz or 175 ml of wine, or 1.5 oz or 44 ml of liquor.
[b] Behaviour Rating Scales

4.2.2 Behavioural measurements

The infant behaviour measures used in this study were assessed at 11 months using two different approaches. The behaviour rating scales (BRS) of the Bayley Scales of Infant Development, 2nd edition (BSID-II), were completed by the infant examiner to measure the infant's orientation/engagement, emotional regulation, and motor quality. BRS scores were coded by two independent examiners for 54 infants. Median inter-rater reliability across these rating scales, which were completed by two different examiners, was 94 % (range 79 - 100 %).

In addition to the BRS scores, video recordings of infant behaviour during the administration of the Mental Development Index (MDI) of the BSID-II were coded on a second-by-second basis for inattention and non-elicited activity using The Observer© software (Noldus Information Technology Inc., Leesburg, VA, USA). Data on inattention and non-elicited activity were obtained for 159 and 156 infants, respectively; missing data were due to technical failure (poor quality video image or bad framing). Both the duration (% of examination time) and rate (number of events/minute) of the attention and activity indicators were compiled. Inattention was defined as the proportion of time or rate per minute the infant looked away from the test material and tester during the administration of the MDI (Plusquellec et al., 2007). Non-elicited activity was defined as the proportion of time or rate per minute the infant moved or wriggled. Behavioural coding requires an acceptable inter-observer agreement (usually 80 %). An agreement was defined as both observers coding the

same behaviour within a 2-second window. Looking transitions (attention) of 81 children and activity of 96 children were coded by two independent research assistants periodically during the coding process. Inter-observer agreement was high; kappa, a statistic that measures inter-observer reliability by adjusting for the probability of an agreement occurring by chance, was 0.9 for inattention and of 0.8 for activity (a value of 1 indicates perfect agreement).

4.2.3 Exposure assessment

Infant blood PCB-153 profiles were generated using a previously validated PBPK modeling framework (Verner *et al.*, 2009). Briefly, PBPK modeling is a mathematical representation of physiological processes governing the absorption, distribution, metabolism and excretion of chemicals in the organism. The model used herein consisted of two tissue compartment networks (maternal and infant) linked by both placental and lactational transfer. The conceptual representation of this model is depicted in Figure 4.1. PCB-153 kinetics within the different compartments were mathematically described with mass-balance differential equations that integrated blood perfusion rates, volume of compartments and tissue:blood partition coefficients based on equations available in the literature that consider mother and infant weight/height, pregnancy history and breast-feeding duration. Because PCB-153 distributes solely in lipids, the lipid composition of tissues, blood and milk was used to derive the partition coefficients. Many parameters, such as the volume of compartments, lipid composition of tissues, daily consumption of breast milk, and fraction of lipids in breast milk were based on time-dependent descriptions that allowed estimation of age- and physiology-specific values in each subject.

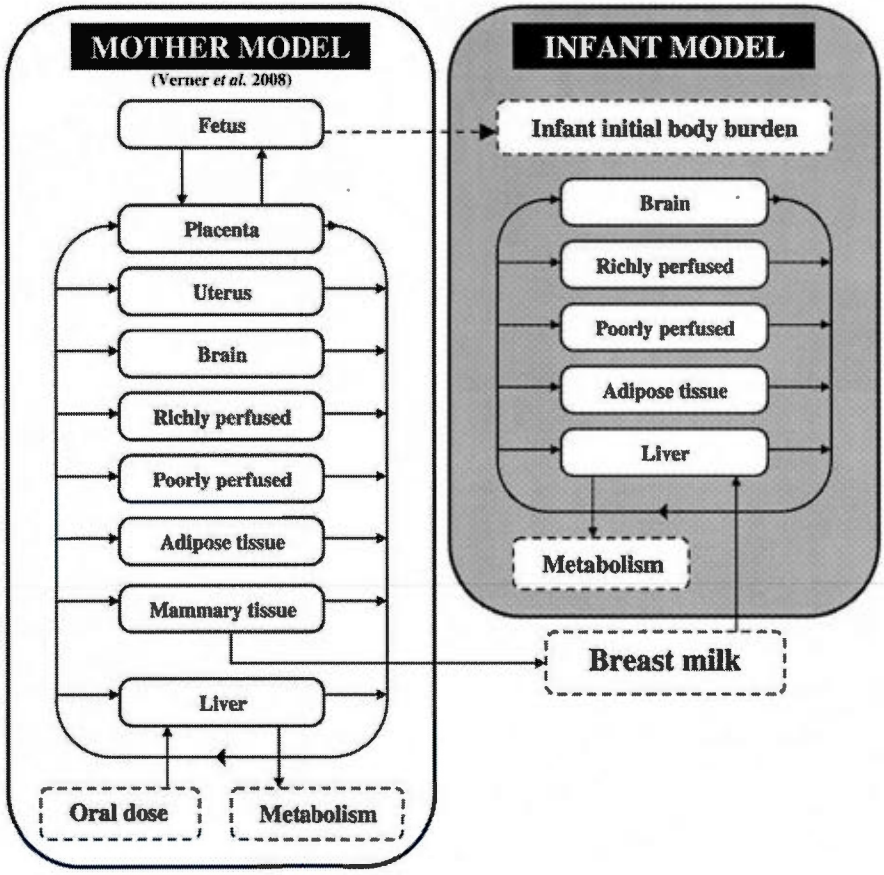

Figure 4.1. Conceptual representation of the physiologically-based pharmacokinetic model for persistent organic pollutants used in this study. Taken fromVerner *et al.* (Verner *et al.*, 2009).

The accuracy of this model in estimating PCB concentrations in infant blood from maternal blood levels was previously examined with data from the same cohort (Verner *et al.*, 2009). Blood PCB levels were obtained at 6 months of age from a subset of the cohort. We performed simulations to estimate 6-month blood PCB-153 levels from maternal blood levels, which we then compared to the measured PCB-153 levels. The analysis was performed by calculating a nonparametric Spearman's rank order correlation coefficient, which examines the relation between two measures based on ordinal scale data. The Spearman's correlation coefficient relating simulated to blood PCB-153 levels obtained from laboratory analyses was high: $r = 0.89$, supporting the validity of the model.

Individualized simulations of PCB-153 toxicokinetic profiles in the infants were based on the mothers' pre-pregnancy weight and height, age at delivery, blood PCB-153 level, date of blood sampling, duration of exclusive breast-feeding, as well as infants' weight and height at delivery and 6 and 11 months. Using the PBPK model and maternal blood PCB-153 level, a constant daily intake (ng/kg/day) was optimized to match blood level at the time of sampling. Infant blood PCB-153 level profile was subsequently simulated for the first 11 months of life. Area under the curve (AUC) of infant blood PCB-153 level was calculated for each month of life. Cord blood PCB-153 level was simulated to provide a surrogate measure of prenatal exposure because cord blood measurements were only available for a subset of the infants. All simulations were carried out using ACSLXtreme software (Aegis Technologies Group, Inc., Huntsville, AL, USA). Exposure profiles were simulated for the 168 infants included in the study.

To avoid selection bias in the statistical analyses, missing model inputs were imputed when sufficient data were available. Missing maternal blood levels (n = 4) were estimated by linear regression based on the strong correlation between maternal and cord blood PCB-153 (r = 0.96). Missing maternal height was replaced by the group average (n = 4). Missing infant weight and height were estimated through linear regression using available measures at other time points (51 infants were missing at least one height or weight measurement).

4.2.4 Control variables

Four sets of potential confounding variables were considered for inclusion in multiple regression models: (1) Maternal characteristics: maternal height, pre-pregnancy weight, age at delivery and parity; (2) Child-rearing environment: primary caregiver's socio-economic status, years of education, marital status (living with partner or not), language at interview (Inuktituk vs. English or French), score on the Peabody Picture Vocabulary Test-Revised and on the Raven Progressive Matrices, mental distress (Indices de Détresse Psychologique – Enquête Santé Québec (IDESP), Préville *et al.*, 1992), crowding (number of residents/room in the infant's home), number of children in the infant's home, score on the Home Observation for Measurement of the Environment (HOME) scale; (3) Birth outcomes:

gestational age, prematurity [< 37 weeks], delivery complications (yes/no); (4) Infant characteristics: gender, age at assessment (approximately 11 months), health status (healthy/sick) at assessment, use of medications at assessment (yes/no), adoption status, daycare (yes/no), number of colds/ear infections during the first year of life, prenatal exposure to other toxicants: cord blood lead and mercury and maternal report of alcohol (number of drinks/day), cigarette smoking (number of cigarettes smoked daily), and illicit drugs (yes/no). In light of recent evidence that polyunsaturated fatty acid composition in cord blood is associated with more optimal infant development (Jacobson et al., 2008), the ratio of docosahexaenoic acid (DHA) to arachidonic acid (AA) was also considered as a potential confounder. We chose the DHA/AA ratio rather than DHA levels because, due to metabolic competition between these fatty acids, the ratio is often considered a better indicator of DHA enrichment (Martinez, 1992). Given that PCB levels are highly correlated with other organochlorines in this population, the latter were not considered in regression models to avoid multicollinearity-related bias in coefficient estimation.

4.2.5 Data analysis

Estimates of cord blood PCB-153 levels and month-by-month AUCs for infant blood PCB-153 levels were log-transformed prior to statistical analyses. One infant was an outlier for behavioural measurements and was removed from analyses since our inquiries revealed that he was sick and under medication at time of testing. Associations between each of these PCB-153 estimates and the 11-month behavioural measures were assessed by multiple linear regression models. All potential confounders that were related to the dependent variable at a p value <0.20 were selected. These variables were then entered individually in series of a regression models for each of the dependent variables, in which each estimate of infant PCB-153 level for a given period was entered at the first step. The order of entry in the model was determined by the strength of the correlation between the potential confounder and the dependent variable. Control variables were retained in the model if their inclusion altered the exposure-effect association at step of entry by $\geq$ 10 %. Statistical analyses were performed using SPSS for Windows (SPSS Inc., Chicago, IL, USA). The β coefficient for PCB-153 from the final regression model measures the strength of the association between exposure

and outcome after statistical adjustment for all control variables that had a meaningful impact on this association.

4.3 Results

4.3.1 Pre- and postnatal PCB-153 levels

Simulated cord blood PCB-153 levels were used to represent prenatal exposure. In this cohort, simulated cord blood levels had a median value of 103 ng/g lipids and ranged from 15 to 706 ng/g lipids. Available measured cord levels had a slightly lower median value of 76 ng/g lipids and ranged from 16 to 551 ng/g lipids ($n = 85$). The temporal pattern of the postnatal PCB-153 level profiles in this population is depicted in Figure 4.2. The Cmax in infant blood for the first 11 months of life had a median value of 241 ng/g lipids and ranged from 25 to 2142 ng/g lipids. There were substantial inter-individual differences in infant blood PCB-153 levels across the first year of life, as illustrated by the examples in Figure 4.3. The maximum blood concentration (Cmax) was reached at birth for bottle-fed infants and at the end of the breast-feeding period for breast-fed infants. The correlation between the cord blood levels and month-by-month postnatal AUCs decreased across the first year from r = 0.96 to 0.45 for the 1st and 11th months, respectively, reflecting the increasing influence of lactational exposure on infant PCB body burden.

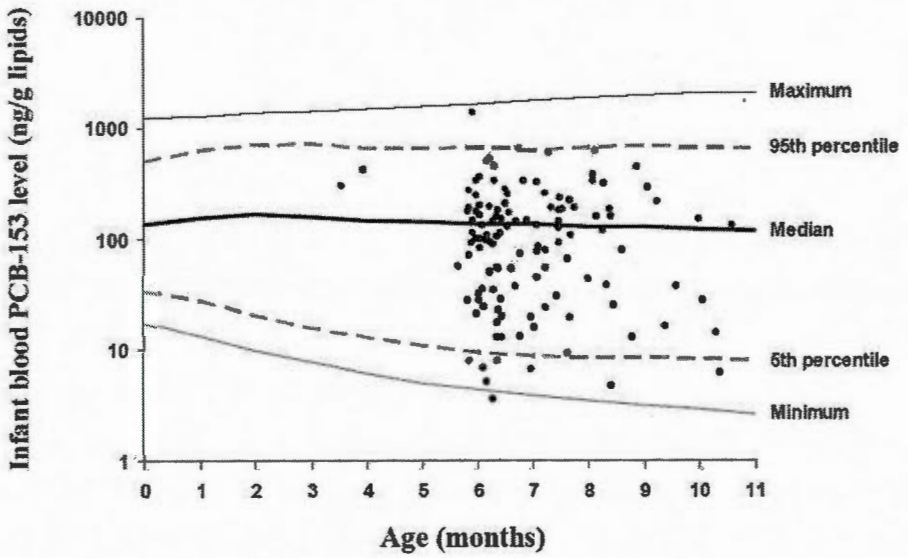

Figure 4.2. Temporal pattern of simulated infant blood PCB-153 levels (ng/g lipids) across the first 11 months of life

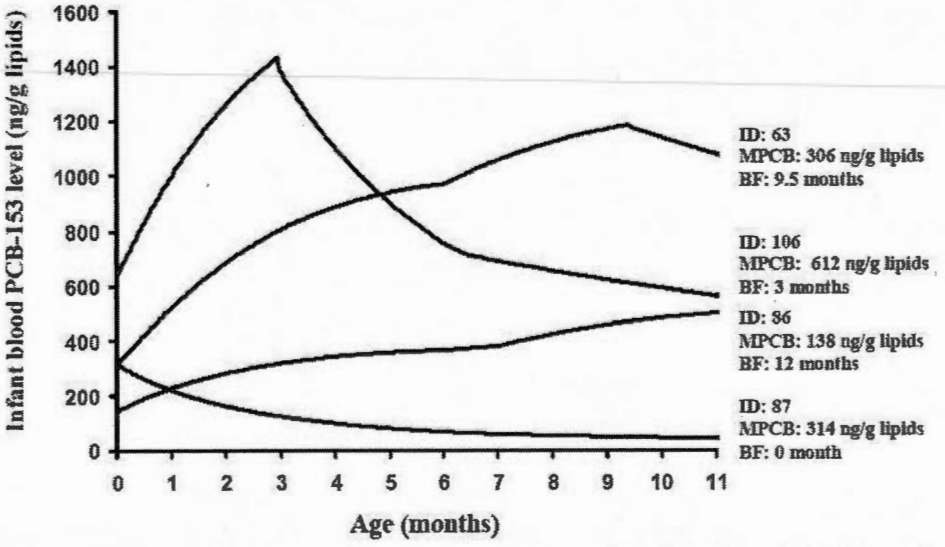

Figure 4.3. Toxicokinetic profiles of four infants (identified by dyad ID) with different input parameters in this study. Maternal blood PCB-153 levels (MPCB) and breast-feeding duration (BF) are provided for each dyad.

4.3.2 Association between PCB levels and behavioural indicators

We used Spearman's correlation coefficients to screen initially for associations between the PCB-153 level estimates and behavioural outcomes (Table 4.2). Significant associations were found with BRS motor quality and observer-coded inattention and non-elicited activity. Whereas the correlation for inattention was strongest with prenatal exposure, postnatal AUCs were the best predictors of non-elicited activity duration and motor quality, with a peak association found for infant blood PCB-153 during the 4th month. The highest time-dependent correlation with these three behavioural indicators was consistently stronger than the correlation obtained with Cmax, which represents the level that can be reached at any time during the first 11 months of life (including cord blood level for bottle-fed infants). This finding provides additional support for the hypothesis that the effects of PCB-153 differ depending on the timing of the exposure.

Multiple linear regression models were constructed to predict each of the behavioural outcomes identified in the screening analyses as a function of prenatal exposure and the month-by-month AUCs, after control for potential confounding variables (Figure 4.4). Significant associations were found between cord blood PCB-153 level and inattention, and month-by-month AUCs during the 2- to 10-month period with non-elicited activity duration. A 10-fold increase in cord blood PCB-153 level was associated with a 3.4 % increase in inattention duration (Figure 4.5). For each 10-fold increase in PCB-153 AUC during the 4th month, non-elicited activity duration increased by 3.8 %. Because regression estimates can be biased by influential points, we re-ran the regression analyses omitting the children with residuals greater than 2 standard deviations. After removing those cases, significance disappeared only for the association between the AUC for the 2nd month and non-elicited activity duration. Although the associations of PCB-153 with BRS motor quality fell short of significance after controlling for confounders, the profile of β values over time was similar to what was seen for non-elicited activity duration.

Table 4.2. Spearman's correlations between blood PCB-153 levels and behavioural indicators at 11 months of age.

			Area under the curve of infant blood PCB-153 concentration (months) [1]											
Behavioral indicators	**n**	**Cord[2]**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**Cmax[3]**
BRS orientation/engagement	168	0.086	0.065	0.042	0.030	0.029	0.026	0.016	0.006	-0.009	-0.014	-0.018	-0.021	0.018
BRS motor quality	167	0.017	0.062	0.130	0.165*	0.166*	0.155*	0.149	0.136	0.124	0.111	0.103	0.099	0.092
BRS emotional regulation	168	0.054	0.059	0.072	0.077	0.072	0.065	0.063	0.053	0.046	0.039	0.033	0.032	0.046
BRS total	167	0.064	0.051	0.033	0.019	0.015	0.006	-0.002	-0.012	-0.026	-0.034	-0.036	-0.037	-0.004
Inattention duration (%)	153	0.205*	0.164*	0.098	0.066	0.059	0.055	0.051	0.047	0.041	0.039	0.033	0.033	0.127
Inattention rate (nb/min)	153	0.121	0.102	0.075	0.066	0.061	0.057	0.054	0.054	0.054	0.051	0.048	0.049	0.087
Non-elicited activity duration (%)	150	0.135	0.163*	0.209*	0.241**	0.250**	0.246**	0.241**	0.232**	0.219**	0.206*	0.193*	0.182*	0.181*
Non-elicited activity rate (nb/min)	150	0.010	0.043	0.100	0.135	0.137	0.141	0.135	0.130	0.125	0.118	0.112	0.107	0.078

[1] Area under the curve of simulated infant blood lipid-adjusted PCB-153 level during each postnatal month
[2] Simulated cord blood lipid-adjusted PCB-153 level (ng/g lipids)
[3] Maximum simulated infant blood lipid-adjusted PCB-153 level reached during the first 11 months (ng/g lipids)
* $p < 0.05$
** $p < 0.01$

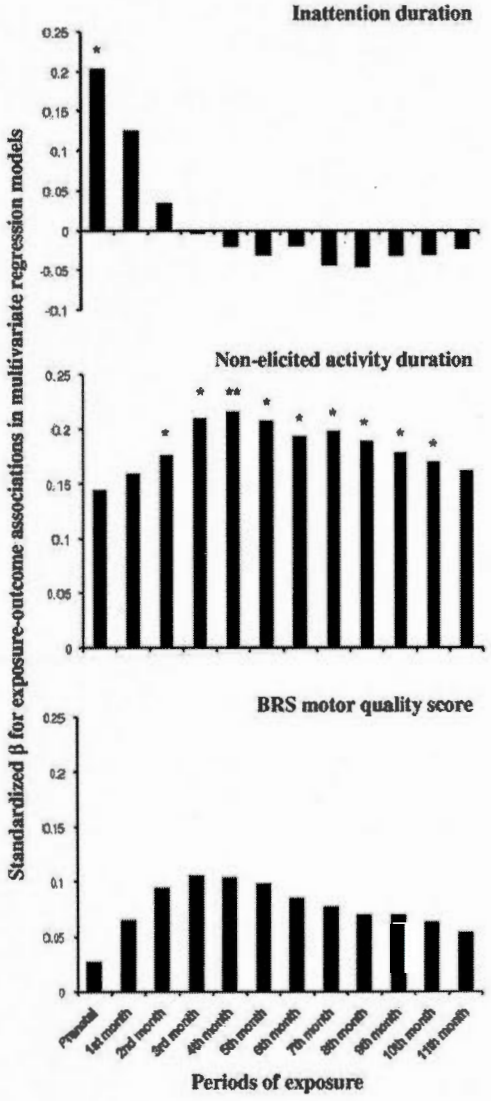

Figure 4.4. Standardized β values for associations between estimates of infant blood PCB-153 levels and behavioural indicators from linear regression analyses. The time points for which each of the control variables was included are indicated in parentheses (0 = prenatal; 1-11 = postpartum months). For non-elicited activity duration: DHA/AA ratio (0-11), prematurity (0-2), health status at examination (0-1), primary caregiver socioeconomic status (1), parity (7). For BRS motor quality: age at examination (0, 2-11), socioeconomic status (0), primary caregiver score on Raven Progressive Matrices (0-11), HOME score (0, 6-11), dha/aa ratio (0, 1, 11), illicit drug consumption during pregnancy (1). For inattention duration: maternal distress (1-11), alcohol consumption at conception (1-11), parity (2-3, 6-11), complications at delivery (2-4), age at examination (3-6, 9-11), cord lead level (1-11), number of cigarettes per day during pregnancy (6, 9-11), sex (7-10), maternal language at interview (2-6, 11). * $p < 0.05$ ** $p < 0.01$

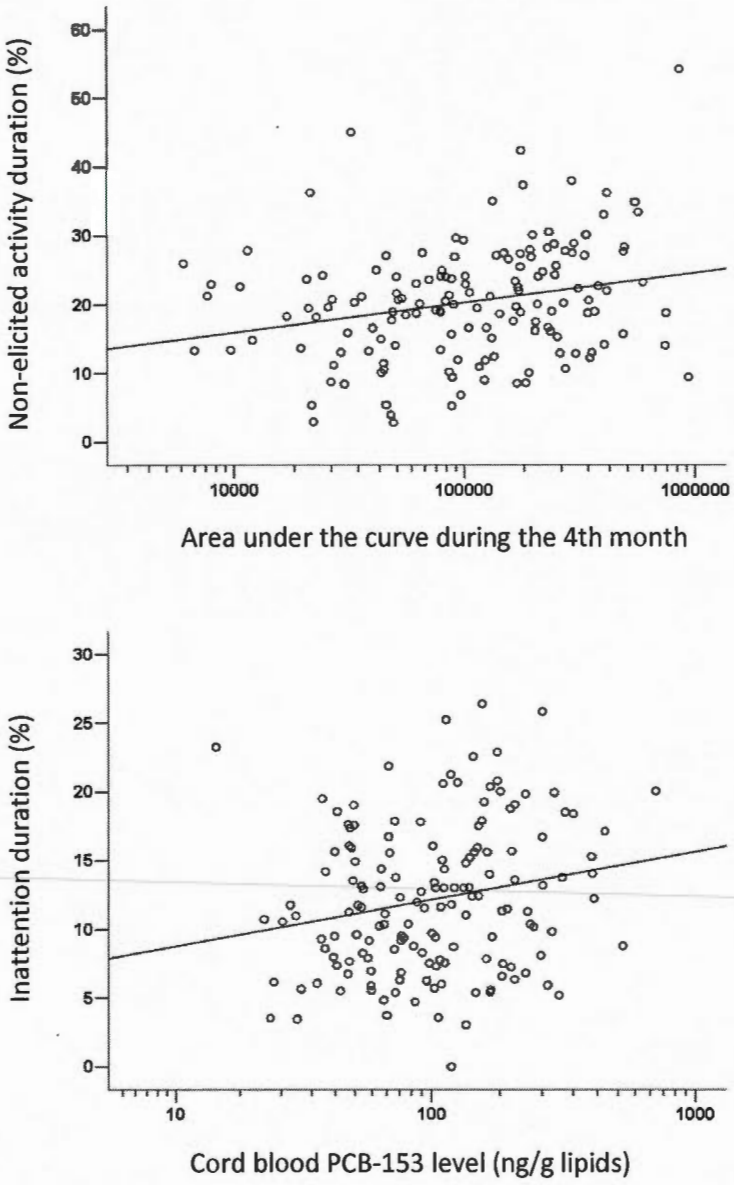

Figure 4.5. Associations between levels of PCB-153 and behavioural indicators of inattention and non-elicited activity (without adjustment for confounders). Note: *x*-axes are in log scale.

4.4 Discussion

Because most epidemiologic studies to date have relied on blood and/or milk samples obtained on a single occasion to characterize postnatal exposure to PCBs, previous studies have not been able to assess critical windows of susceptibility during the first year of life. Using PBPK modeling to estimate infant toxicokinetic profiles, we investigated whether pre- and postnatal levels of PCB-153 (informed by estimates of cord blood levels and month-by-month infant PCB-153 AUCs) alter behavioural function at 11 months of age.

The results of this study suggest that different aspects of neurobehavioral function—activity level, assessed by non-elicited activity, and inattention—have different periods of vulnerability to PCBs. Prenatal exposure was associated with a significant increase in inattention at 11 months but was not related to non-elicited activity. This pattern is consistent with results recently published by Sagiv *et al.* (2008), which demonstrated that PCBs in cord blood are associated with poorer quality of alertness but do not relate to activity as assessed with the Neonatal Behavioral Assessment Scale 2 weeks after birth. Data from other studies suggest that prenatal PCB-induced deficits in attention are maintained throughout childhood, up to 11 years of age (Jacobson et Jacobson, 2003). Attention in infancy has also been found associated with prenatal lead exposure (Plusquellec *et al.*, 2007). Our findings add to the converging evidence of the vulnerability of attention to intrauterine neurotoxic exposures.

For non-elicited activity, our data suggest a postnatal window of vulnerability, with a peak association for PCB-153 AUC during the 4^{th} month of life. During this period there is extensive myelination of certain brain regions (Marsh *et al.*, 2008), a process believed to be affected by PCBs, as PCB-118 has been shown to alter human progenitor cell differentiation into oligodendrocytes (myelinating cells in the central nervous system) in a thyroid-like manner (Fritsche *et al.*, 2005). In the Michigan study, postnatal exposure (defined in terms of child's blood PCB level at 4 years of age) was related to decreased activity level at 4 years of age (Jacobson *et al.*, 1990), while prenatal exposure was associated with increased impulsivity at 11 years (Jacobson et Jacobson, 2003). Increased activity as measured on the Werry-Weiss-Peters Activity Scale at 3 to 12 years of age was also observed in prenatally

exposed children born to women who consumed PCB-contaminated rice oil during the *Yu-Cheng* incident in Taiwan (Chen *et al.*, 1994). Postnatal exposure was not assessed in that study because the *Yu-Cheng* mothers were advised not to breast-feed. In the Oswego study at 4.5 years of age, prenatal PCB exposure was related to decreased response inhibition, which is crucial to the ongoing regulation of behaviour (Stewart *et al.*, 2003), and at 8 years to increased impulsivity (Stewart *et al.*, 2005). Thus, previous studies suggest that prenatal PCB exposure is associated with activity level, and most report increased activity levels in childhood. Our study, which is the first to examine windows of vulnerability during the postnatal period, is also the first to suggest that postnatal PCB exposure may increase PCB-related impairment in the child's ability to control his/her activity. Our data showing that cord PCB-153 in itself was not significantly related to non-elicited activity suggest that additional postnatal exposure through an extended period of susceptibility may have been required to trigger the observed effect on 11-month infant activity in this cohort.

As noted above, PCB-153, the most prevalent PCB congener, was used as a surrogate to represent the highly stable mixture of PCB congeners found in the Arctic (Ayotte *et al.*, 2003; Muckle *et al.*, 2001). The very high correlations between PCB-153 and chlorinated pesticides, that stem from both their common source of exposure in this cohort and their long half-life in the body, prevented their inclusion as control variables in statistical analyses. Given the strong intercorrelations among these contaminants (Muckle *et al.*, 2001), the observed associations with PCB-153 may conceal complex toxicological interactions (addition, synergy and antagonism) and, therefore, caution should be exercised when evaluating the contribution of PCBs to these associations. Nevertheless, cohort studies with different exposure sources (and consequently different environmental mixtures) have consistently reported similar PCB-induced neurodevelopmental outcomes and thus support a significant contribution of PCBs to these behavioural endpoints (Boucher *et al.*, 2009). Our results are also consistent with the behavioural deficits observed in experimental laboratory studies with monkeys and rats exposed to PCBs postnatally (Holene *et al.*, 1998). We have carefully considered the principal nonchlorinated contaminants—mercury and lead—as well as other prenatal exposures, including alcohol and smoking, as potential confounders. All of the associations between PCB exposure and infant behaviour reported here were significant

after either (a) ruling out these potential confounders because their influence on the relation of PCB exposure to the endpoints examined was no more than minimal or (b) statistical adjustment for their influence.

Given that the observed effects involved only small differences in infant inattention and activity, these effects must be considered subclinical. The notion of subclinical toxicity comes from studies showing that children's exposure to lead can impair intelligence and behavioural function even in the absence of clinically evident symptoms of toxicity. The assumption is that there is a dose-dependent continuum of toxic effects, in which clinically obvious effects have subclinical counterparts (Landrigan, 1989). The subclinical toxicity of lead has been confirmed in numerous prospective epidemiologic studies (Chiodo *et al.*, 2004; Lanphear *et al.*, 2005; Surkan *et al.*, 2007). In the structured setting in which the BSID-II is administered, our visual inattention measure appeared to provide a natural assessment of an inability to maintain focus and alertness over time, which is usually referred to sustained attention (Mirsky *et al.*, 1991). Sustained attention is a complex psychological construct that is under the control of higher cortical and subcortical centers. Stewart *et al.* (2003) have reported that the relation of prenatal PCB exposure to poorer response inhibition, another aspect of attention, is moderated by corpus callosum size. The neural bases of the effect of PCB exposure on sustained attention have not been examined.

In our study, the association between prenatal PCBs and inattention and particularly between postnatal PCBs and non-elicited activity may be precursors of increased impulsivity in later stages of development. Given the limited predictive validity of most measures of cognitive function in infancy, however, any inferences regarding continuity from infancy to school-age must be made with caution (Becker *et al.*, 2004; Olson *et al.*, 2002). Studies on the capacity of these infant behavioural measures to predict later childhood behaviour as well as associations between PCB levels and later health outcomes in this population are currently underway.

Although we evaluated our behavioural outcomes in relation to multiple PCB-153 level estimates, these findings do not appear to be attributable to chance. Three (38.5 %) of the

eight endpoints were related to PCB exposure. Rather than chance findings, all the correlations with each endpoint are significant across contiguous months, revealing when during development the effects appear to be strongest (Table 4.2). It is of interest that the relation of lactational exposure to PCBs with non-elicited activity shares a similar temporal pattern with the BRS motor quality score despite the limited correlation with the latter endpoint. These converging results confer internal consistency to our findings and suggest a common mechanism of action of PCBs on these two behavioural outcomes.

4.5 Conclusions

The findings from this study provide support for the hypothesis that PCBs can induce different behavioural outcomes when exposures occur during different periods of vulnerability. Our results add to the growing evidence of deficits in attention in children exposed prenatally to PCBs and suggest that heavy postnatal exposure through breast-feeding during the first postpartum year can increase non-elicited activity in infancy. These PCB-induced effects may be of even greater significance for children genetically predisposed to behavioural problems such as attention-deficit hyperactivity disorder. Previous reports of adverse effects from postnatal exposure (e.g., Koopman-Esseboom *et al.*, 1996) have been limited by the difficulties of discriminating the relative contributions of maternal milk PCB level and duration of breast-feeding (Jacobson et Jacobson, 2001). This study is the first to use a sophisticated PBPK model to more definitively identify an adverse neurobehavioral effect from postnatal PCB exposure in a human cohort. It should be noted that this effect might not have been detected even with this methodology at the substantially lower levels of postnatal exposure observed in previous studies. Moreover, given that the effects are subclinical, it should be emphasized that the risk to the child even at the relatively high levels of PCB exposure in this population is unlikely to outweigh the benefits of breast-feeding. Nevertheless, the novel exposure assessment approach used in this study offers a new perspective into windows of susceptibility to persistent organic pollutants, which can help reveal associations that can be difficult to identify using traditional exposure metrics.

Acknowledgments

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CONCLUSION GÉNÉRALE ET PERSPECTIVES

1. Discussion générale des résultats

L'évaluation de l'exposition est souvent une grande faiblesse des études épidémiologiques. Lorsque des mesures biologiques sont effectuées, elles se limitent généralement à un seul échantillon de sang, de lait ou d'urine pour caractériser l'exposition d'un individu. L'interprétation des niveaux de contaminants mesurés dans ces échantillons est souvent déficiente, ce qui gêne grandement les conclusions qui peuvent être tirées de ces études d'envergure. Par exemple, plusieurs études épidémiologiques récentes ont évalué les associations entre l'exposition aux phthalates ou au bisphénol A et une multitude d'effets sur la santé, tels que les mensurations à la naissance (Suzuki *et al.*, 2010b), le comportement des enfants (Braun *et al.*, 2009; Swan *et al.*, 2010) et l'intelligence chez les enfants d'âge scolaire (Cho *et al.*, 2010), en ne se basant que sur un seul échantillon d'urine. La courte demi-vie de ces composés fait en sorte que les niveaux mesurés dans l'urine ne représentent que l'exposition durant les dernières heures avant le prélèvement. Quelques études ont d'ailleurs démontré que la variabilité dans les niveaux de bisphénol A ou de phthalates mesurés dans les échantillons récoltés successivement sur une période de 24 heures est expliquée principalement par la variation dans les échantillons d'une même personne. Ainsi, l'utilisation d'un seul échantillon ne permet pas de tenir compte des variations interindividuelles dans l'exposition et rend toute conclusion quant aux effets sur la santé douteuse (Fromme *et al.*, 2007; Ye *et al.*, 2011; Braun *et al.*, 2011; Preau *et al.*, 2010; Marcus *et al.*, 2010). Pendant ce temps, une multitude d'outils d'évaluation de l'exposition voient le jour en toxicologie, mais ils demeurent tout simplement inconnus des épidémiologistes. L'absence quasi-totale de dialogue entre toxicologues et épidémiologistes est un obstacle important à l'avancement des connaissances en santé environnementale.

L'objectif général de ce projet était d'élaborer des modèles PBPK qui permettent de simuler des profils complets d'exposition interne aux POP et d'intégrer ces modèles dans le cadre d'études épidémiologiques. Dans un premier temps, un modèle PBPK a été mis sur pied pour décrire la cinétique des POP chez les femmes afin de retracer le profil d'exposition interne

des individus enrôlés dans des études épidémiologiques sur le cancer du sein. Ce modèle, qui intègre les différents processus physiologiques importants dans la cinétique des POP, a permis de quantifier l'effet que peuvent avoir différents événements comme les changements de poids et l'allaitement sur les niveaux internes de POP. Des exercices de simulation avec différents scénarios physiologiques (ex : nombre de grossesses, durée d'allaitement) et d'exposition ont aussi permis de faire la démonstration que plusieurs profils d'exposition internes peuvent résulter en une même concentration sanguine au moment du diagnostic, ce qui appuie la remise en question de la présupposition qu'un seul échantillon puisse représenter l'exposition chronique aux POP. Ces résultats ont suscité beaucoup d'intérêt dans la communauté scientifique. L'article présenté au chapitre I a été non seulement publié dans la revue la plus prestigieuse en santé environnementale (*Environmental Health Perspectives*), mais aussi mis en valeur dans un éditorial rédigé par monsieur Nathaniel Mead et publié comme un *Highlight* dans le même numéro de la revue. Alors que je présentais une affiche à la *Society of Toxicology* à Charlotte aux États-Unis, j'ai été approché par D[re] Lizbeth López-Carrillo de l'*Instituto Nacional de Salud Publica* au Mexique afin d'aller présenter mes résultats au congrès de l'*International Society of Environmental Epidemiology* à Mexico et de poursuivre mon séjour au Mexique à titre de chercheur invité à l'institut où elle œuvre. Dans l'ensemble, tout ceci illustre bien la pertinence et l'impact de ce travail qui constitue la première pierre de la présente thèse de doctorat.

Ces travaux ont aussi attiré l'attention de madame Delphine Bachelet et du P[r] Pascal Guénel, deux épidémiologistes basés à l'*Institut National de la Santé et de la Recherche Médicale* (INSERM) à Paris. Ces derniers ont démontré un intérêt à utiliser ce modèle PBPK dans le cadre d'une étude cas-témoins sur le cancer du sein constituée de 2135 femmes françaises dont l'exposition aux PCB a été évaluée à partir d'échantillons sanguins. Une première étape de ce projet visait à évaluer la relation entre les concentrations sanguines en PCB-153 dans le sang des femmes au moment du diagnostic et les concentrations sanguines simulées à l'aide du modèle PBPK pour les décennies antérieures. Tel que présenté dans l'article du chapitre II, les résultats ont permis d'évaluer la représentativité de tels échantillons sanguins et, tout comme un chapitre I, d'identifier l'allaitement comme facteur pouvant réduire la capacité d'un échantillon à représenter l'exposition passée. La publication de cet article dans

un journal de très bonne renommée, *Cancer Epidemiology, Biomarkers and Prevention*, est un gage de reconnaissance de notre travail. La deuxième étape du projet vise quant à elle à évaluer les associations entre l'exposition aux PCB à différentes périodes de la vie telle qu'estimée à l'aide du modèle PBPK et l'incidence du cancer du sein dans la même population. Au moment d'écrire ces lignes, l'équipe de l'INSERM travaille à la rédaction de l'article dans lequel seront rapportés les résultats de cette étude novatrice. Les résultats préliminaires de cette étude laissent croire qu'une exposition aux PCB durant la puberté, telle que simulée à l'aide du modèle PBPK, réduirait les risques de développer un cancer du sein. Cette association inverse était beaucoup plus forte que celle obtenue avec les mesures sanguines au moment du diagnostic, un résultat qui supporte l'hypothèse voulant que le tissu mammaire soit plus susceptible aux atteintes chimiques durant certaines périodes de la vie. Cette observation concorde avec des expériences *in vitro* effectuées au laboratoire du P[r] Michel Charbonneau où des agonistes du récepteur Ah, dont certains congénères de PCB font partie, ont freiné la prolifération de cellules cancéreuses mammaires humaines (Pittet *et al.*, article en préparation). Ces observations originales démontrent clairement le potentiel de la modélisation PBPK dans les études épidémiologiques sur le cancer du sein.

Bien que l'utilité de ce modèle PBPK dans les études épidémiologiques ait été démontrée, certains aspects mériteraient d'être explorés plus en profondeur. Tout d'abord, la capacité du modèle à estimer l'exposition antérieure avec précision n'a pas encore été mise à l'épreuve. La validation complète du modèle nécessiterait le recrutement de femmes desquelles seraient prélevés des échantillons sanguins à plusieurs moments de la vie et pour lesquelles une estimation de la dose journalière serait effectuée afin de comparer les résultats des simulations aux valeurs expérimentales. Il faut réaliser qu'une telle étude est pratiquement impossible à entreprendre en raison de limitations logistiques évidentes (suivre des femmes pendant toute leur vie, mais surtout être capable de mesurer leur exposition à tous les moments). En revanche, si des mesures sanguines répétées sur une certaine période de la vie de femmes devenaient disponibles, il serait envisageable d'effectuer une validation partielle pour vérifier la capacité de notre outil à prédire les concentraitons sanguines en POP à divers points dans le temps. Encore une fois, il serait essentiel de pouvoir évaluer correctement les variations possibles du niveau d'exposition, ce qui est un grand défi.

Une autre avenue de recherche serait la caractérisation des paramètres physiologiques d'intérêt au sein de différentes populations. Le modèle est, jusqu'à maintenant, basé sur des descriptions physiologiques moyennes calculées à partir de données chez les caucasiens. La disponibilité de données propres à la population à l'étude permettrait probablement d'estimer l'exposition interne aux POP avec une précision supérieure. Dans le même ordre d'idées, les paramètres physiologiques ne varient pas seulement en fonction de l'âge, du poids corporel et de la taille. Une athlète de 30 ans pesant 65 kg et mesurant 1,70 m a probablement une physiologie très différente de celle d'une femme ayant les mêmes mensurations mais n'étant pas active, et ce, notamment au niveau du volume du tissu adipeux. Par exemple, certaines mesures d'impédance (conduction électrique du corps) dans le cadre des études épidémiologiques permettraient d'estimer le volume du tissu adipeux de façon plus précise qu'en ne se basant que sur le poids, la taille et l'âge des individus. L'allaitement est aussi un paramètre du modèle qui pourrait bénificier de données plus exhaustives. Dans le modèle actuel, la description de l'allaitement est basée sur le temps écoulé depuis la naissance de l'enfant ainsi que sur le poids de ce dernier. Par contre, une variation dans la production de lait peut être observée dans la population (Arcus-Arth *et al.*, 2005). En identifiant les déterminants du volume de lait produit par jour (ex : nombre de grossesses antérieures, durée d'allaitement total [Ingram *et al.*, 1999]) et en quantifiant leur influence sur ce paramètre, il serait possible de décrire plus précisément la production de lait chez chacune des femmes en fonction de leurs caractéristiques individuelles. Enfin, les processus d'élimination mériteraient aussi d'être investigués plus en détails. Premièrement, le métabolisme hépatique des POP dépend de la quantité d'enzyme et de leur efficacité. Les différences dans la génétique (ex : les polymorphismes des cytochromes p450), le mode de vie des femmes (ex : cigarette, alcool) et l'ontogénie (variations dans l'efficacité enzymatique en fonction de l'âge), des facteurs qui n'ont pas été pris en compte dans le modèle jusqu'à présent, peuvent avoir un effet sur la biotransformation des POP. Par ailleurs, les POP peuvent être excrétés par les fèces puisqu'ils se partitionnent entre le tissu intestinal et son contenu en fonction de la composition lipidique des deux milieux (Moser et McLachlan, 2001). Une étude chez des victimes de l'exposition accidentelle au TCDD à Seveso en Italie a d'ailleurs démontré que la consommation d'un gras non-absorbable, l'olestra pendant 38 jours peut réduire la demi-vie

du TCDD de plusieurs années (Geusau *et al.*, 1999). L'élimination par les fèces peut donc varier d'un individu à l'autre, notamment en fonction des habitudes alimentaires. L'identification des déterminants de ces deux modes d'élimination et leur intégration dans le modèle PBPK permettraient de réduire l'incertitude liée à ces processus et ainsi accroître la précision du modèle.

Alors que le modèle PBPK décrivant la cinétique des POP chez les femmes a connu un certain succès chez les épidémiologistes, tel que discuté plus tôt, c'est le modèle développé pour l'exposition aux POP par l'allaitement qui a suscité le plus d'intérêt (chapitre III). En ayant accès aux données d'une cohorte d'Inuits du nord du Québec, j'ai pu vérifier la concordance entre les concentrations sanguines en POP simulées à l'aide du modèle PBPK et les concentrations sanguines mesurées chez les enfants d'environ six mois. Cette étape de validation du modèle et les informations qui peuvent être tirées des simulations (ex : concentration sanguine maximale) sont deux éléments majeurs qui ont séduit à la fois les toxicologues et les épidémiologistes. Tout comme le modèle PBPK décrivant la cinétique des POP chez les femmes, l'article sur l'élaboration et la validation de ce modèle a été publié dans le journal *Environmental Health Perspectives*.

Les profils toxicocinétiques des enfants obtenus lors de l'exercice de validation ont ensuite été utilisés afin d'évaluer les associations entre l'exposition aux PCB à différentes périodes de développement et des indicateurs d'attention de l'activité spontanée chez les enfants inuits de 11 mois (chapitre IV). L'utilisation du modèle PBPK a permis de distinguer deux fenêtres de susceptibilité aux PCB, soit une période prénatale et une période postnatale. Alors que l'exposition prénatale était associée à une réduction de l'attention des enfants, l'exposition postnatale (spécialement durant le quatrième mois) était associée à une augmentation de l'activité spontanée. C'est grâce à l'utilisation du modèle PBPK qu'il a été possible d'identifier une période de susceptibilité postnatale aux atteintes neurotoxiques des PCB, un résultat d'importance qui n'aurait pas pu être révélé en utilisant les approches traditionnelles d'évaluation de l'exposition. Ces résultats laissent croire que certains processus de neurodéveloppement ayant lieu après la naissance (ex : myélinisation, synaptogénèse) peuvent être perturbés par une exposition aux PCB par l'allaitement. Cette étude aura donc

des retombées dans l'analyse du risque de l'exposition aux PCB ainsi qu'aux autres substances neurotoxiques qui sont transportées dans le lait maternel.

Malgré la grande concordance entre les concentrations sanguines simulées par le modèle PBPK et celles mesurées chez les enfants, il demeure que le modèle PBPK décrivant le transfert mère-enfant de POP ne réussit pas à expliquer 100 % de la variation dans les niveaux sanguins mesurés chez les enfants. Plusieurs avenues sont envisageables afin de calibrer ce modèle. Tel que mentionné pour le modèle de la femme, l'obtention de données physiologiques propres aux populations à l'étude permettrait une meilleure estimation des paramètres influençant la cinétique des POP, tels que le volume du tissu adipeux et la composition lipidique des organes. La légère surestimation des niveaux sanguins dans l'exercice de validation présenté au chapitre III laisse croire que la description de l'allaitement surestime peut-être le volume de lait produit par jour dans cette population. Une surestimation semblable a été observée lors des analyses préliminaires de validation qui ont été entreprises récemment dans une cohorte d'enfants slovaques. La caractérisation du volume de lait produit dans ces populations augmenterait sans doute la précision du modèle. En termes pratiques, quelques femmes pourraient être sélectionnées afin qu'elles pèsent leur enfant avant et après les tétées au cours d'une période de 24 heures à quelques reprises durant les mois d'allaitement. Ces données renseigneraient sur le volume ingéré par unité de poids corporel en fonction du temps écoulé depuis la naissance et des différents déterminants comme le nombre de grossesses antérieures. Enfin, l'allaitement mixte entre aussi en jeu dans l'estimation de l'exposition chez les enfants. Afin d'estimer l'exposition durant cette période où l'enfant est nourri à la fois avec du lait maternel et d'autres sources (ex : lait en formule), le pourcentage que représente l'allaitement dans l'alimentation de l'enfant devrait être recueilli dans les questionnaires épidémiologiques. En explorant ces diverses avenues de recherche, il serait possible de considérer plus de paramètres propres aux individus enrôlés dans les études épidémiologiques et, en conséquence, d'obtenir des profils d'exposition interne plus précis.

Pour la première fois, la modélisation PBPK a été intégrée dans des études épidémiologiques afin d'estimer l'exposition aux POP durant différents moments de la vie, notamment durant

des périodes où les individus pourraient être plus susceptibles aux atteintes chimiques. En tenant compte des phénomènes physiologiques ayant une influence sur la cinétique des POP, les modèles PBPK permettent de bien caractériser la variabilité interindividuelle dans les profils d'exposition et, en conséquence, de détecter des effets liés à l'exposition aux POP dans les études épidémiologiques. De façon plus générale, les résultats novateurs qui ont été générés dans ces études manifestent la nécessité qu'un dialogue prenne place entre les toxicologues et les épidémiologistes afin d'aborder les problématiques en santé environnementale en utilisant les connaissances complémentaires propres à ces deux domaines. Les avancées scientifiques qui découleront de telles collaborations permettront de mieux caractériser le risque posé par les composés toxiques et d'établir des stratégies afin de réduire les méfaits causés par une exposition à ces contaminants.

2. Perspectives de recherche

Dans la foulée de l'application du modèle PBPK sur le transfert mère-enfant au sein d'études épidémiologiques, divers projets en cours et futurs illustrent bien l'impact des réalisations présentés dans cette thèse. Ainsi, des travaux de modélisation seront effectués pour deux autres cohortes de naissance basées dans le nord du Québec. Les enfants de ces cohortes ont été suivis pendant plusieurs années (cinq ans dans un cas, 10 ans dans l'autre), ce qui permettra d'évaluer la persistance ou la latence des effets suite aux expositions pré- et postnatales. Le vaste éventail de tests psychométriques qui leur ont été administrés permettra aussi d'identifier avec plus de confiance les domaines cognitifs, moteurs et comportementaux altérés par une exposition aux PCB. Les simulations ont déjà été effectuées dans une de ces deux cohortes, et les associations entre ces estimations de l'exposition et des traits comportementaux ainsi que la santé de la vision sont présentement à l'étude dans les laboratoires respectifs des docteurs Pierrich Plusquellec et David Saint-Amour.

Lors de la présentation des premiers résultats sur les effets des expositions prénatales et postnatales aux PCB sur l'attention et l'activité des enfants (chapitre IV) au congrès de l'*International Society of Environmental Epidemiology* à Dublin, D^re^ Monica Guxens du laboratoire du P^r^ Jordi Sunyer a lancé les bases d'une collaboration pour la simulation de

l'exposition postnatale aux PCB, au DDE et à l'hexachlorobenzène dans sept cohortes basées en Espagne. Ces cohortes regroupent plus de 2500 enfants des régions de Gipuzkoa, Menorca, Granada, Valencia, Sabadell, Asturias et Ribera d'Ebre qui ont été examinés à environ un an et cinq ans pour plusieurs indicateurs de neurodéveloppement et immunitaire. Les associations entre l'exposition postnatale aux PCB, au DDE et à l'hexachlorobenzène telle qu'estimée à l'aide du modèle PBPK seront évaluées par Mireia Gascon au *Centre de Recerca en Epidemiologia Ambiental* à Barcelone en Espagne.

Dans la même lignée, une étude sera entreprise avec une cohorte d'enfants nés près d'un site contaminé aux PCB en Slovaquie. Cette étude, sous la direction des professeurs Tomas Trnovec (Slovak Medical University) et Irva Hertz-Picciotto (University of California at Davis), a la particularité d'avoir prélevé et analysé des échantillons de sang de plusieurs enfants à l'âge de six mois, 16 mois et 45 mois. Ces données permettront de valider et calibrer le modèle pour des prédictions allant jusqu'à l'âge de quatre ans. Des données préliminaires ont démontré que le modèle explique un grand pourcentage de la variabilité dans les niveaux sanguins des enfants jusqu'à 22 mois. Les profils d'estimation simulés pour ces enfants serviront par la suite à évaluer l'effet d'une exposition postnatale aux PCB sur le développement cognitif et psychomoteur des enfants.

Le modèle PBPK sera aussi utilisé dans une cohorte d'enfants nés près d'un site contaminé aux PCB, mais cette fois-ci au États-Unis. Cette cohorte de naissance constituée de 788 enfants nés à New Bedford au Massachusetts a été mise sur pied par D^re^ Susan Korrick de la *Harvard School of Public Health/Brigham and Women's Hospital*. Un point d'intérêt majeur dans cette étude est que les enfants ont été examinés pour une multitude de fonctions comportementales, cognitives et psychomotrices à six mois, à huit ans et à 15 ans, permettant ainsi d'évaluer la persistance ou la latence des effets des PCB. Alors que les analyses effectuées jusqu'à présent dans cette étude sont basées uniquement sur des niveaux mesurés dans le sang au cordon ombilical, l'utilisation du modèle PBPK contribuera significativement aux analyses en estimant l'exposition postnatale.

Pour leur part, la P^re^ Brenda Eskenazi et le D^r^ Jonathan Chevrier de *l'University of California at Berkeley* ont initié une collaboration afin d'étudier les effets de la pulvérisation d'insecticides à l'intérieur des maisons en Afrique du Sud sur le neurodéveloppement et endocrinien des enfants. La modélisation PBPK portera sur l'exposition postnatale au DDT/E chez les enfants enrôlés dans l'étude. Les informations tirées des simulations et des niveaux sanguins mesurés chez les enfants de un an permettront de distinguer la contribution des différentes voies d'exposition (i.e., lait maternel, transfert intra-utérin, exposition environnementale) à la charge corporelle de l'enfant. En identifiant les sources les plus déterminantes des niveaux sanguins de l'enfant, des stratégies d'action visant à réduire l'exposition pourront être mises en place plus aisément.

Alors que les niveaux en PCB et DDT dans les échantillons humains et environnementaux ont décliné significativement aux États-Unis et au Canada depuis leur retrait du marché dans les années '70, d'autres produits de synthèse avec des propriétés semblables ont suivi et ravivent la problématique des POP. C'est notamment le cas des PBDE, des retardateurs de flamme lipophiles et persistants dont la structure chimique partage des similarités frappantes avec les PCB. La production de la majorité des congénères de PBDE a été stoppée récemment, mais leur présence dans les articles rembourrés (ex : canapés, matelas, sièges d'automobile) et les produits électroniques (ex : ordinateurs, téléphones cellulaires) achetés avant cet arrêt de production fait en sorte que nous y serons exposés pendant encore plusieurs années. Ceci est inquiétant puisque plusieurs études épidémiologiques suggèrent que ces composés altèrent diverses fonctions dont le développement du cerveau (Roze *et al.*, 2009; Herbstman *et al.*, 2010; Gascon *et al.*, 2011), le système reproducteur (Harley *et al.*, 2010) et le système endocrinien (Herbstman *et al.*, 2008; Chevrier *et al.*, 2010). L'étude CHAMACOS, sous la direction de la P^re^ Brenda Eskenazi de l'*University of California at Berkeley*, vise à évaluer les effets de ces contaminants sur le développement cognitif et psychomoteur des enfants. À cette fin, une collaboration en cours vise à tracer des profils d'exposition postnatale aux PBDE pour les enfants enrôlés dans cette cohorte de naissance. De plus, une estimation de l'exposition prénatale d'enfants recrutés à l'âge de neuf ans dans une nouvelle cohorte (CHAMACOS II) sera effectuée à l'aide du modèle PBPK et des

niveaux sanguins mesurés au moment de l'inclusion dans l'étude. L'étude des effets d'une exposition postnatale aux PBDE sera la première en ce genre.

Enfin, un autre projet sur la problématique des PBDE pourrait voir le jour dans les prochaines années. Ce projet ferait suite à un article d'extrapolation *in vitro* - *in vivo* de la neurotoxicité des PBDE rédigé en collaboration avec la P^re^ Ellen Fritsche de l'*Institut für umweltmedizinische Forschung gGmbH an der Heinrich-Heine Universität* (Appendice A). Cet article fait la revue de la littérature pertinente sur la neurotoxicité des PBDE, établi une stratégie afin d'extrapoler les relations dose-réponses *in vitro* à la situation *in vivo* et propose des pistes de recherche afin de permettre l'utilisation des données *in vitro* en analyse du risque. L'approche proposée repose entre autres sur l'estimation des niveaux en PBDE dans le cerveau des enfants exposés par l'allaitement et par la poussière à l'aide de la modélisation PBPK afin de comparer ces niveaux aux concentrations intracellulaires menant à une neurotoxicité *in vitro*.

En terminant, les rapprochements disciplinaires découlant des travaux présentés dans cette thèse et les collaborations futures avec divers groupes oeuvrant en épidémiologie environnementale montrent que les objectifs visés dans cette thèse ont été pleinement atteints.

APPENDICE A

IN VITRO NEUROTOXICITY DATA IN HUMAN RISK ASSESSMENT OF POLYBROMINATED DIPHENYL ETHERS (PBDES): OVERVIEW AND PERSPECTIVES

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Abstract

Polybrominated diphenyl ethers (PBDEs) are flame retardants routinely detected in samples of cord blood and breast milk. Concerns have been raised with regard to the toxicity of both prenatal and postnatal exposures towards the developing nervous system. Although there is an increasing body of literature on the disruption of brain cell functions by certain PBDE congeners *in vitro*, some challenges have yet to be tackled to enable the translation of *in vitro* findings into their *in vivo* counterparts. In this paper, we review findings on the PBDE neurotoxicity in human cells and discuss the research gaps to be addressed. Moreover, we propose a scheme for the incorporation of *in vitro* data in human risk assessment, namely through i) the determination of *in vitro* cell benchmark levels; ii) the consideration of uncertainties in establishing equivalency between the *in vitro* and the *in vivo* tissue benchmark levels (e.g., chronic vs. acute exposure, interactions with other chemicals); and iii) relating tissue benchmark levels to surrogate levels of internal exposure. Alongside the assessment of brain dosimetry following exposure to PBDEs, *in vitro* neurotoxicity data provide a unique opportunity to evaluate the risks of prenatal and early life exposures on children neurodevelopment.

Key words: Polybrominated Diphenyl Ethers (PBDEs), Neurotoxicity, Risk Assessment, *In vitro - In vivo* Extrapolation, Exposure Assessment.

1. Introduction

Polybrominated diphenyl ethers (PBDE) are flame retardants that have been used in several consumer products such as electronic equipment, textiles, upholstered furniture, and plastics since the 1970s (Gouin *et al.*, 2005; Rahman *et al.*, 2001). Over time, PBDEs leach from household products into the indoor environment. Consequently, these chemicals are routinely detected in samples of house dust and air, thereby revealing a potential for human exposure (Frederiksen *et al.*, 2009). These compounds are lipophilic (Li *et al.*, 2008b) and bioaccumulate in living organisms (Kuo *et al.*, 2010). In addition, PBDEs released in the environment biomagnify in the food chain (de Wit *et al.*, 2010) and, as a result, are frequently detected in consumer products (Frederiksen *et al.*, 2009). Their presence in human cord blood and breast milk samples (Frederiksen *et al.*, 2009) raises concerns regarding their potential adverse effects on foetus and infant development.

There are 209 possible PBDE congeners that differ in their degree of bromination and the position of bromine atoms on the diphenyl ether backbone. PBDEs have been marketed under three chemical formulations with different congener mixtures: the penta-, the octa- and the deca-PBDE (Rahman *et al.*, 2001). The penta and octa technical mixtures have been phased out recently whereas the deca formulation is still widely produced and used. Nevertheless, humans remain exposed to congeners that constituted the penta and octa mixtures since they are still present in products bought before their use was restricted and persist in the indoor environment.

Several studies assessed PBDE neurotoxicity in animals, cell lines and humans. Epidemiological studies on PBDEs and infant neurodevelopment are very limited but suggest that prenatal exposure may impair certain brain processes (Herbstman *et al.*, 2010; Roze *et al.*, 2009). To date, no human study investigating the association between postnatal exposure and neurodevelopment has been published. On the other hand, animal studies on pre- and postnatal exposure to PBDEs reported neurobehavioural alterations such as hyperactivity, impaired learning and decreased habituation (reviewed by Costa et Giordano, 2007). Results

from *in vitro* studies on human brain cells clearly suggest that PBDEs can disrupt several cell functions (Table A.1), which could be mechanistically relevant to the *in vivo* alterations mentioned above.

One major challenge in assessing the risk of pre- and postnatal exposure to PBDEs is translating *in vitro* benchmark levels into their *in vivo* counterparts. To enable such extrapolations, several pharmacokinetic and pharmacodynamic parameters need to be thoroughly considered. In this paper, we review current findings on PBDE neurotoxicity in human cells and discuss how these observations can be incorporated to achieve scientifically sound human risk assessment.

Table A.1. Neurotoxic effects of PBDEs in human cell cultures.

Endpoint	Test	Cell type	Congener	Treshold	Reference
Cytotoxicity	MTT reduction	132-1N1 astrocytoma	PBDE-99	25 μM	Madia et al. (2004)
		SH-SY5Y neuroblastoma	PBDE-47	8.3 μM	He et al. (2008)
		SH-SY5Y neuroblastoma	PBDE-47	5 μM	He et al. (2009)
		SK-N-SH neuroblastoma	DE-71	8.9 μM	Yu et al. (2008)
		SK-N-MC neuroblastoma	PBDE-47	5 μM	Tagliaferri et al. (2010)
			PBDE-99	10 μM	Tagliaferri et al. (2010)
	LDH release	SH-SY5Y neuroblastoma	PBDE-47	8.3 μM	He et al. (2008)
		SH-SY5Y neuroblastoma	PBDE-47	5 μM	He et al. (2009)
		SK-N-SH neuroblastoma	DE-71	22.1 μM	Yu et al. (2008)
		human neuroprogenitor cells	PBDE-47/-99	>10 μM	Schreiber et al. (2010)
Apoptosis	TUNEL	132-1N1 astrocytoma	PBDE-47	50 μM	Madia et al. (2004)
	PI staining	SH-SY5Y neuroblastoma	PBDE-47	8.3 μM	He et al. (2008)
		SK-N-SH neuroblastoma	DE-71	6.4 μM	Yu et al. (2008)
ROS production	DCFH-CA	SH-SY5Y neuroblastoma	PBDE-47	4.2 μM	He et al. (2008)
DNA damage	Olive tail moment	SH-SY5Y neuroblastoma	PBDE-47	5 μM	Gao et al. (2009)
		SH-SY5Y neuroblastoma	PBDE-47	2.1 μM	He et al. (2008)
	DNA in tail	SH-SY5Y neuroblastoma	PBDE-47	5 μM	Gao et al. (2009)
		SH-SY5Y neuroblastoma	PBDE-47	16.6 μM	He et al. (2008)
	8-OHdG	SH-SY5Y neuroblastoma	PBDE-47	10 μM	Gao et al. (2009)
Chromosome abnormalities	NDI, MNBNC, NPB	SH-SY5Y neuroblastoma	PBDE-47	4.2 μM	He et al. (2008)
	MNI	SH-SY5Y neuroblastoma	PBDE-47	8.3 μM	He et al. (2008)

*MTT: (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide; LDH: Lactate dehydrogenase; TUNEL: TdT-mediated dUTP Nick-End Labeling; PI: Propidium idodide; DCFH-CA: 2',7'-Dichlorodihydrofluorescein diacetate; 8-OHdG: 8-hydroxy-2'-deoxyguanosine; NDI: Nuclear division index; MNBC: Micronucleated binucleate cells; NPB: Nucleasmic bridges; Micronuclei.

2. Current knowledge on PBDE exposure and neurotoxicity

PBDE exposure in humans

Human exposure to PBDEs has been documented in numerous biomonitoring and epidemiological studies. Exposure in adults is thought to occur mainly through the ingestion of house dust, and to a lesser extent through the consumption of contaminated food (Lorber, 2008). The presence of PBDEs in cord blood samples reveals that the foetus is also exposed throughout the prenatal period (Frederiksen *et al.*, 2009). Postnatally, exposure is maintained as PBDEs are transferred from the mother to the nursing infant through breast milk. Additionally, children are extensively exposed through the ingestion of house dust, which was reported to be twice that of adults on a body weight basis (EPA, 1997).

Prenatal exposure to PBDEs is usually assessed by dosing the chemicals in maternal blood during pregnancy or in cord blood. In light of the Frederiksen et al. (2010) study on PBDE partitioning between maternal and umbilical cord blood, which showed that PBDE levels in these two matrices are highly correlated, both measures appear likely to provide a good approximation of foetal exposure. PBDE levels in maternal and cord blood worldwide have been reviewed recently by Frederiksen et al. (2009). Concentrations in samples from North America were strikingly higher than those reported in Europe and Asia.

Although levels in human breast milk have been extensively biomonitored (Frederiksen *et al.*, 2009), little information is available on actual postnatal levels in infant blood. Blood concentrations in the early postnatal period are of particular concern since this is a time-window during which the developing brain undergoes synaptogenesis, myelinization and apoptosis (Marsh *et al.*, 2008). In a study on pooled serum samples from 8132 Australians, Toms et al. (2008) reported that levels in children below 4 years of age to be on average two-fold higher than those measured between 5 and 15 years of age, and four-fold higher than levels observed in individuals above 16 years of age. The peak blood ΣPBDE level in the same population was reached at 2.6-3 years of age (Toms *et al.*, 2009).

PBDE toxicokinetics

Because of their lipophilic properties, PBDEs are thought to be mainly distributed in body lipids. Levels of many congeners such as PBDE-47 and PBDE-99 were found to be in the same concentration range in human blood, liver and adipose tissue when expressed on a lipid basis (Ericson Jogsten *et al.*, 2010; Covaci *et al.*, 2008; Meironyte Guvenius *et al.*, 2001). However, little is known about the partition of PBDEs in brain lipids. Tissue distribution in adult Sprague-Dawley rats exposed orally to a mixture of PBDEs displayed a similar congener pattern in the brain, adipose tissue, kidney and lung for most congeners (Huwe *et al.*, 2008), but the congener PBDE-209 did not distribute in most tissues including the brain and was found primarily in the liver and plasma. To our knowledge, PBDEs have yet to be measured in human brain tissue. On the other hand, tissue levels of polychlorinated biphenyls (PCBs), similar to PBDEs in terms of physicochemical properties and persistence, have been measured in adipose, liver and brain tissues of human stillborns (Lanting *et al.*, 1998). On a lipid basis, the concentration of ΣPCB (sum of PCB-118, -138, -153, and -180) was the highest in adipose tissue. Levels in the liver and brain lipids were lower with liver/adipose and brain/adipose ratios of 0.8 and 0.2. Similarly, Dewailly et al. (1999) found brain lipid-ajusted PCB levels to be 2- to 10-fold lower than those measured in adipose tissue lipids. This lower concentration in brain lipids is thought, by the same authors, to be the result of the higher polarity of brain lipids compared to adipose and liver tissue lipids.

Beyond simple partitioning in body lipids, brain dosimetry following exposure to PBDEs can be estimated by means of pharmacokinetic modeling. To date, only one physiologically based pharmacokinetic (PBPK) model describing the kinetics of PBDEs has been published (Emond *et al.*, 2010). This PBPK model was used to simulate PBDE-47 kinetics in Sprague-Dawley rats following either oral or intravenous dosage. PBDE distribution in the brain was described as a diffusion-limited process with a brain:blood partition coefficient of 3. Simulated tissue levels, including brain levels, following a single oral dose of 1 μmol/kg were in good agreement with measured levels.

Findings in epidemiology

Only two epidemiological studies have been published on the neurotoxicity of PBDEs. The first study was conducted by Roze et al. (2009) in a prospective cohort of 62 Dutch mother-infant dyads. They reported several associations between maternal blood organohalogens levels measured during the 35th week of pregnancy and their 5-6 year-old children performance on a battery of motor, cognitive and behavioural tests. The levels of certain PBDE congeners were correlated with diminished fine manipulative abilities (PBDE-154) and decreased sustained attention (PBDE-47), while they were also associated with a better coordination (PBDE-47, -100), visual perception (PBDE-153), and behaviour (PBDE-47, -99, -100). After controlling for socio-economic status, Home Observation for Measurement of the Environment (HOME) and gender, maternal PBDE levels were correlated with decrements in fine manipulative abilities (PBDE-154), sustained attention (PBDE-47, -99, 100) and verbal memory (PBDE-153), and with better selective attention (PBDE-47) and behaviour (PBDE-47, -99, -100). These effects were observed for low levels (median in ng/g lipids [maximum]) of PBDE-47 (0.9 [6.1]), PBDE-99 (0.2 [2.1]), PBDE-100 (0.2 [1.4]), PBDE-153 (1.6 [19.7]) and PBDE-154 (0.5 [3.5]). These results should however be interpreted with caution given that statistical analyses were performed on a small sample of 62 mother-infant dyads.

A recently published study carried out by Herbstman et al. (2010) also suggested that PBDEs can impair infant neurodevelopment. This longitudinal cohort study included 329 mothers who gave birth in New York (US). Prenatal exposure to PBDEs was assessed through measurements in cord blood samples. Median levels of PBDEs (ng/g lipids [maximum]) were approximately one order of magnitude above the concentrations reported by Roze et al. (2009): PBDE-47 (11.2 [613.1]), PBDE-85 (0.7 [16.6]), PBDE-99 (3.2 [202.8]), PBDE-100 (1.4 [71.9]), PBDE-153 (0.7 [28.9]), PBDE-154 (0.6 [11.1]), PBDE-183 (0.6 [2.8]). Infant neurodevelopment was assessed at 12, 24 and 36 months by administrating the Bayley Scales of Infant Development, Second Edition (BSID-II). Children were also administered the Wechsler Preschool and Primary Scale of Intelligence, Revised Edition (WPPSI-R) at the age of 48 and 72 months. After adjusting for a variety of control variables, negative associations

were observed with the mental and psychomotor development indexes of the BSID-II, as well as with the full, verbal and performance IQ components of the WPPSI-R. The latter assessment at 48 and 72 months is of particular concern as these measures are important predictors of subsequent academic performance. A limitation of this study is that other organic neurotoxicants such as polychlorinated biphenyls (PCBs) were not measured, and consequently not considered in statistical analyses although co-exposure is likely. These results have yet to be replicated in other large birth cohorts.

Overall, these studies indicate that prenatal exposure to PBDEs at environmental levels may impair infant development. The associations found at much lower levels in the European study than those reported in the US suggest the existence of a continuum of subtle effects in the low exposure range and prompt further investigation. To date, no study assessed the potential associations between infant neurodevelopment and postnatal exposure through breast-feeding and extensive dust ingestion, a critical period for brain development.

In vitro studies using human cancer cells

Several studies have found that PBDEs induce various neurotoxic effects in immortalized human brain cell lines (Table A.1). One common finding across studies is cytotoxicity induced by short-term exposures to PBDE-47 (He *et al.*, 2009; He *et al.*, 2008; Gao *et al.*, 2009; Tagliaferri *et al.*, 2010), PBDE-99 (Madia *et al.*, 2004; Tagliaferri *et al.*, 2010), or the technical mixture DE-71 (Yu *et al.*, 2008). The lowest concentrations leading to cytotoxicity, as measured by MTT reduction or release of lactate dehydrogenase, ranged from 5 to 25 μM. Using non-linear regressions to fit the experimental data on the Hill function, Tagliaferri et al. (2010) estimated Benchmark Doses (10% effect on viability for the lowest 95 % CI extreme) of 3.1 and 6.8 μM for PBDE-47 and PBDE-99, respectively. In addition, apoptosis was observed in human cells exposed to PBDE-99 (Madia *et al.*, 2004), PBDE-47 (He *et al.*, 2008) and the commercial penta-PBDE mixture DE-71 (Yu *et al.*, 2008). As observed by He et al. (2008) and Gao et al. (2009), PBDE-47 can also induce the production of reactive oxygen species (ROS), DNA damage and chromosome abnormalities in SH-SY5Y neuroblastoma cells (Table A.1).

In vitro study using human neuroprogenitor cells

A recent study by Schreiber et al. (2010) investigated the neurotoxicity of PBDE-47 and PBDE-99 in primary human neuroprogenitor cells (hNPCs). These cells grow as 3D neurospheres and undergo several neurodevelopmental processes such as proliferation, differentiation and migration *in vitro* (Moors *et al.*, 2009). In addition, these cells were shown to be sensitive to methylmercury (Moors *et al.*, 2007) and polychlorinated biphenyls (PCBs; Fritsche *et al.*, 2005), two known developmental neurotoxicants. Results from this study provided strong evidence for PBDE-induced alteration of migration and differentiation of neuroprogenitor cells into neurons and oligodendrocytes (Table A.2). After 7 days of differentiation, 16.3 % of cells exposed to the lowest PBDE-99 medium concentration (0.1 μM) adopted a neuron phenotype compared to 26.6 % in control cells. Both PBDE-47 and PBDE-99 caused a concentration-dependent decrease in neural progenitor cell migration that reached significance at a concentration of 1 μM. Cell viability and proliferation were not affected by PBDEs within a range of 0.1 to 10 μM. This *in vitro* model, which undergoes three of the human brain development milestones, seems to be more sensitive than immortalized cells as effects were detected at lower medium PBDE concentrations. Overall, the lowest concentrations in medium leading to altered human cell functions were 0.1 μM of PBDE-99 and 1 μM of PBDE-47. According to the accumulation assay described in this study, levels in cells would be approximately 60-fold higher than medium initial concentration (6 μM of PBDE-99 and 60 μM of PBDE-47). These levels are equal to approximately 3.4 μg/g cells of PBDE-99 and 28.8 μg/g cells of PBDE-47. Lipids composition of the neuroprogenitor cells was not measured and therefore these levels were not expressed on a lipid basis.

Table A.2. Effects of PBDE-47 and PBDE-99 on human neuroprogenitor cells within a range of 0.1 to 10 µM medium concentrations (Schreiber *et al.*, 2010).

	Threshold concentration (% of control)	
Neurotoxic endpoint	PBDE-47	PBDE-99
Viability	-	-
Proliferation	-	-
Differentiation		
Oligodendrogenesis	1 µM (68.4)	1 µM (47.4)
Neurogenesis	1 µM (63.2)	0.1 µM (61.3)
Migration	1 µM (80.2)	1 µM (71.6)
Calcium signalling	-	-

3. Perspectives in the use of in vitro data for human risk assessment

In vitro dose-response assessments present a unique opportunity to refine human risk assessment for neurotoxic compounds such as PBDEs. They allow screening for a wide range of concentrations and cell responses, and they can be performed directly on human material. Although studies on animal cells can yield valuable information on mechanisms of action (e.g. brain cells from knockout rodents) and allow complementary *in vivo* experiments, the uncertainty related to interspecies extrapolation limits their use in human risk assessment.Translating results from *in vitro* assays (in human brain cells) into *in vivo* dose-response relationships (in obtainable blood or tissue samples) could provide valuable information to assess whether environmental, occupational or accidental exposures can lead to hazardous internal levels (see Figure A.1).

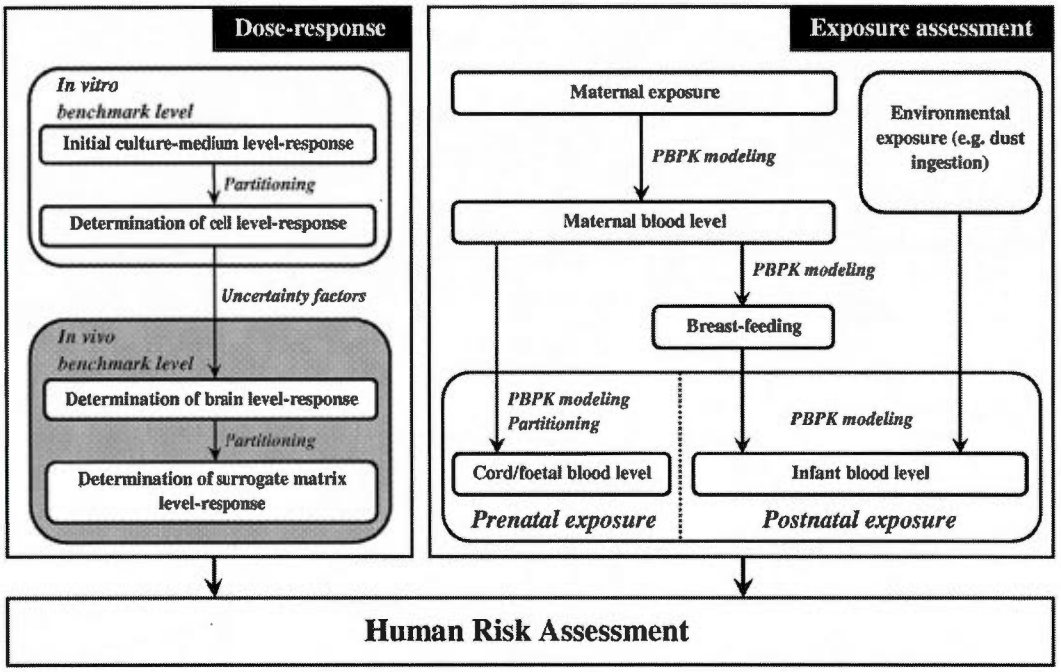

Figure A.1. Proposed scheme for the incorporation of *in vitro* PBDE neurotoxicity data into human risk assessment. First, *in vitro* benchmark levels based on initial medium concentration need to be expressed on a cell-level basis through appropriate measurements or the use of partition coefficients. Subsequently, cell level-response relationships need to be translated into tissue level-response by considering related uncertainty factors. Brain level-response can then be transformed into surrogate matrix level-response, which could be compared to either biomonitoring data or internal levels estimated through toxicokinetic modeling.

Dose-response: Determination of cell level-response

To extrapolate *in vitro* dose-response relationships to *in vivo* toxicodynamics, culture medium concentrations first need to be transformed into cell concentrations. The bioaccumulation of PBDEs in human brain cells *in vitro* depends on several factors. Mundy et al. (2004) showed that PBDE-47 accumulation in primary cell cultures of rat neocortex increased with time, medium concentration, medium protein composition, and volume of medium. Results from Kodavanti et al. (2005) in rat cerebellar granule neuronal cultures also suggested that bioaccumulation depends on the experimental conditions and differs between PBDE congeners. Finally, Schreiber et al. (2010) showed that exposure of hNPCs to 1 μM ^{14}C-

BDE-47 for 7 days resulted in a brain cell concentration of 61.16 ± 6.34 µM. In these experimental conditions, the ratio between final cell concentration and initial medium concentration was approximately 60. Because experimental settings influence PBDE accumulation in brain cells, reported accumulation factors cannot be extrapolated to other exposure conditions and, therefore, cell concentration must be measured in every study.

Dose-response: Translating in vitro brain cell level-response into in vivo foetal/infant brain level-reponse

Translating *in vitro* brain cell level-response into *in vivo* brain tissue level-response requires the inclusion of uncertainty factors. To date, *in vitro* studies assessed effects within time-frames varying from 1 to 14 days. These experiments are much shorter than the 9-month *in utero* and lactational exposure periods and might not allow the detection of effects that require more time to reach significance at low concentrations. Also, *in vitro* assays may only partially represent the mechanisms of action leading to neurotoxicity *in vivo* if indirect physiological processes such as the disruption of hormone regulation are also involved. For example, PBDEs have been shown to lower blood thyroid stimulating hormone (TSH) in pregnant women (Chevrier *et al.*, 2010). This could in turn affect brain cell functions, in addition to the direct effects of PBDEs. Another important source of uncertainty is the interaction between the different PBDE congeners and other xenobiotics. Additive or synergistic toxicodynamic interactions could lead to neurotoxic effects at lower concentrations of individual congeners. On the other hand, there are counterpart uncertainties related to the fact that *in vitro* experiments do not consider possible brain compensation that could occur *in* vivo. These uncertainty factors may yield discrepancies between *in vitro* brain cell benchmark levels and *in vivo* brain levels leading to neurotoxicity and should therefore be taken into account.

Dose-response: Extrapolation of inferred in vivo brain tissue level-response into surrogate tissue level-response

Using toxicokinetic considerations, *in vivo* brain tissue level-response relationships inferred from *in vitro* data can theoretically be translated into obtainable surrogate level-response relationships. Surrogates of internal exposure to persistent organic pollutants include blood, adipose tissue and breast milk. In most epidemiological/toxicological studies, infant blood or breast milk lipid-adjusted concentration is used to assess postnatal exposure, and maternal blood or cord blood lipid-adjusted concentration is used as an indicator of prenatal exposure. Similarities in PBDE levels in blood, adipose tissue and liver lipids suggest that tissue distribution is mostly driven by lipid composition. This pattern is consistent with the distribution of other lipophilic chemicals (Haddad *et al.*, 2000). Thus, to transform inferred brain tissue benchmark levels into blood lipid benchmark levels, estimated whole brain levels of PBDE need first be transformed into brain lipid-adjusted levels by considering brain tissue lipid composition, which varies throughout development with a 1.5 % lipid content (w:w) at 20 weeks of gestation, 2.6 % lipid content at birth and 6.1 % at 18 months (White *et al.*, 1991). Levels in blood lipids can then be estimated from lipid-adjusted brain concentration. Although studies on PCBs suggest that lipid-adjusted levels may be lower in the brain compared to adipose and liver tissues, it seems reasonable to assume that PBDE levels in foetal/infant blood and brain lipids will be in the same range. This assumption could be verified using either paired samples of blood and brain or through *in vitro* partitioning experiments.

When studying the prenatal neurotoxicity of PBDEs, one could estimate the equivalency between PBDE levels in foetal blood lipids (or cord blood lipids which is equivalent) and lipid-adjusted maternal blood levels. To estimate foetal blood levels from maternal blood levels, placental transfer of different congeners needs to be accounted for. In a study of paired samples of maternal and cord blood plasma, Frederiksen et al. (2010) observed that transport across the placenta was inversely associated with the degree of bromination. The cord to maternal blood lipid-adjusted level ratios for PBDE-28, PBDE-47 and PBDE-153 were 1.3, 1.0 and 0.4, respectively. Transport of PBDE-209 across the placenta was not observed in this study but is known to occur since this congener has been measured in cord blood samples (Antignac *et al.*, 2009; Gomara *et al.*, 2007).

Exposure assessment and risk assessment

Once *in vivo* benchmark levels in surrogate tissues are established, human exposure assessment must be carried out to evaluate potential exposure scenarios and their resulting internal levels to determine if there is a risk of neurodevelopmental toxicity (Figure A.1). Because the brain undergoes important development both pre- and postnatally, exposure during both periods is relevant to studies on PBDE neurotoxicity. While maternal or cord blood levels may be adequate to represent foetal exposure, postnatal levels are much more difficult to characterize. Internal levels over the first years of life are the result of both exposure (e.g. breast-feeding) and physiology (e.g. growth). These concurrent processes have highly influential effects on the toxicokinetics of lipophilic compounds and limit the reliability of current blood and breast milk sampling methods to thoroughly assess children exposure profile.

Toxicokinetic tools, such as physiologically-based pharmacokinetic (PBPK) models, have been developed to simulate the fate of xenobiotics in the body following oral, dermal and inhalation exposures. PBPK modeling incorporates all parameters relevant to absorption, distribution, metabolism and excretion of xenobiotics in the exposed organism to characterize temporal profiles of internal exposure (Krishnan et Anderson, 2001). Such models describing the lactational transfer of persistent organic pollutants like PCBs in humans have been published (Redding *et al.*, 2008; Verner *et al.*, 2009). The validated PBPK model described in Verner et al. (2009) simulates infant internal levels of different persistent organic pollutants through breast-feeding across the first year of life. Should the data on postnatal blood PBDE levels be available, these models could be extended to PBDEs to estimate likely early life exposures through breast-feeding. Using PBPK modeling, we previously demonstrated that exposure to PCBs during a specific postnatal time window was associated with decreased ability to control activity in infants, a finding that would not have been captured by current sampling methods unless infant blood was sampled at this precise period of development (Verner *et al.*, 2010).

4. Research needs

Although there is an increasing body of literature on *in vitro* PBDE neurotoxicity, certain crucial areas of research have yet to be explored before *in vitro* data reach their full potential in human risk assessment. One of the major gaps in PBDE *in vitro* neurotoxicity is the lack of dose-response data at the low concentration end of the curve. *In vitro* experiments often use concentrations several orders of magnitude above the highest expected target organ levels. In addition, they mostly rely on statistical analyses based on data distribution to determine thresholds of neurotoxicity (e.g. Student's t-test). These approaches are hampered by several limitations related to their high dependency on neurotoxin concentration selection and sample size (Davis *et al.*, 2010). Moreover, they do not take into consideration the shape of the dose-response curve. Other approaches such as the benchmark dose, which relies on fitting dose-response models to *in vitro* data rather than hypothesis testing, allow the characterization of excess risk at low concentrations without the limitations of threshold approaches. Non-linear regression can be used to fit continuous data (e.g. cell migration, differentiation) to saturation-type dose-response models (Gaylor *et al.*, 1998), a method that allows prediction of adverse effects at any concentration within the range of experimental conditions. It can also account for experimental variability to derive benchmark doses for the lowest extreme of the confidence interval.

Neurotoxicity cannot be defined solely in terms of cell level-response. Both the timing and duration of exposure may also play a critical role in the alteration of neurodevelopment by PBDEs. In the Schreiber et al. (2010) study on human neuroprogenitor cells, PBDEs were found to reduce cell migration and differentiation into neurons and oligodendrocytes. Cell migration and neurogenesis occur mainly during the prenatal period whereas oligodendrogenesis extends well after birth. These findings suggest that exposure to PBDEs during both the pre- and postnatal periods may harm the developing brain. To thoroughly investigate the impact of postnatal exposure on neurodevelopment, *in vitro* studies should also evaluate the impact of PBDEs on myelination, synaptogenesis and apoptosis. Likewise, *in vitro* studies should evaluate PBDE neurotoxicity over extended periods of exposure.

Although cell cultures may be difficult to sustain over a long period, this may allow the identification of effects that require chronic exposure.

One of the remaining obstacles to incorporating *in vitro* data in human risk assessment is that only a limited number of neurotoxic endpoints have been investigated so far. The development and functioning of the central nervous system is the result of a highly diverse array of general cell functions (e.g. energy metabolism, glucose uptake, calcium homeostasis) and specific processes (e.g. electrical activity, neuronal-glial interactions, synaptogenesis) that could all potentially be targets for neurotoxic compounds like PBDEs (Bal-Price et al., 2010a). The increasing availability of human models to study these different endpoints sets the ground for a more in-depth examination of PBDE neurotoxicity. Among these endpoints, neuronal network's electrical functionality could be evaluated using human neuroprogenitor cells grown on microelectrode arrays. These platforms allow the evaluation of neuron spontaneous and evoked electrical activity in the presence of a suspected neurotoxicant (Yla-Outinen et al., 2010). Also, metabolomics and genomics approaches that were recently developed for rat cells could be applied to human *in vitro* models to both identify and quantify alterations induced by PBDEs (Bal-Price et al., 2010b). The impact of PBDEs on a number of critical neuronal-glial interaction endpoints (e.g., oligodendrocyte and astrocyte swelling, disruption of intercellular signalling) should also be assessed (LoPachin et Aschner, 1993). Obviously, it will be impossible to screen for PBDE effect on every single brain function. Advances in quantitative structure-activity relationship (QSAR) models of neurotoxicity could provide valuable information that would help direct the selection of endpoints to be assessed.

Another issue in PBDE *in vitro* neurotoxicology assays is the lack of data on interactions among congeners and other environmental contaminants. Tagliaferri et al. (2010) provided evidence for a synergistic interaction between PBDE-47 and PBDE-99 on human neuroblastoma cell viability. In another study, PBDE-47-induced cell death in human neuroblastoma was increased by PCB-153 (He *et al.*, 2009). DNA damage and production of reactive oxygen species (ROS) induced by PBDE-47 was also shown to be facilitated by simultaneous exposure to PCB-153 (Gao *et al.*, 2009). Because humans are exposed to

mixtures of PBDEs rather than individual congeners, more research is needed to assess interactions among congeners found in blood and tissue specimens. PBDE mixtures used in *in vitro* assays should ideally represent biologically plausible congener profiles as documented in biomonitoring studies. *In vitro* data on individual congener and mixtures could be used to develop new algorithms to sum PBDE congeners measured in epidemiological studies based on both concentrations and estimated neurotoxic equivalency factors.

Furthermore, although very limited, PBDE metabolism to different metabolites is known to occur. Hydroxylated metabolites are found in blood of humans (Athanasiadou *et al.*, 2008), including fetal blood (Qiu *et al.*, 2009). A study with human hepatocytes provided evidence for PBDE-99 hydroxylation to 2,4,5-tribromophenol, monohydroxylated pentabromodiphenyl ethers and an unidentified tetrabrominated metabolite (Stapleton *et al.*, 2009). It has been shown that PBDE metabolites can have a higher potency than parent compounds at binding to thyroid hormone receptors (Li *et al.*, 2010). Future experiments on human brain cells should thus include predominant PBDE metabolites to assess their neurotoxicity.

5. Conclusion

There is growing evidence that development exposure to PBDEs can lead to neurotoxic responses in humans. To reduce the uncertainties related to *in vitro-in vivo* extrapolation, *in vitro* studies will need to address cell responses at lower concentrations through the use of benchmark approaches, assess neurotoxicity over prolonged periods of exposure and screen for potential interactions among PBDE congeners, metabolites, and other environmental contaminants. Results from such studies and comparison with dose-reponse relationships observed *in vivo* will help assess the validity of *in vitro* data and, consequently, derive validity criteria. Together with the use of appropriate exposure assessment tools, data from *in vitro* studies will enhance our understanding of PBDE effects on the developing nervous system and allow the achievement of scientifically sound human risk assessment.

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