FRÉDÉRIC DALLAIRE

INFECTIONS ET EXPOSITION AUX ORGANO-CHLORÉS CHEZ LES ENFANTS DU NUNAVIK

Thèse présentée
à la Faculté des études supérieures de l'Université Laval
dans le cadre du programme de doctorat en épidémiologie
pour l'obtention du grade de Philosophiae Doctor (Ph.D.)

FACULTÉ DE MÉDECINE
UNIVERSITÉ LAVAL
QUÉBEC

MARS, 2006

Résumé

Les enfants inuits du Nunavik ont une exposition prénatale à certaines substances organochlorées immunotoxiques plusieurs fois supérieures à celle d'enfants du sud du Québec. Cette exposition tend à diminuer dans le temps mais demeure significative et pourrait altérer certaines fonctions de leur système immunitaire. Nous avons calculé que la concentration moyenne de biphenyls polychlorés (BPC) dans le sang de cordon ombilical des nouveau-nés inuits a diminué de près de 8 % par année entre 1994 et 2001. Plusieurs substances peuvent moduler les fonctions du système immunitaire et il existe une grande variété de méthodes pour en mesurer l'effet. Parmi celles-ci, une variation de l'incidence d'infections aiguës dans une population donnée constitue une mesure valide et cliniquement significative. Afin de mesurer l'impact de cette exposition prénatale sur l'incidence d'infections respiratoires aiguës, nous avons révisé les dossiers médicaux de deux cohortes d'enfants du Nunavik (n = 199 et n = 354). Un calcul de l'incidence d'infections respiratoires à partir de la revue des dossiers pendant les cinq premières années de vie a montré une fréquence d'otites moyenne aiguës (OMA), d'infections des voies respiratoires inférieures (IVRI) et d'hospitalisations pour infections respiratoires plus élevée au Nunavik comparativement à la majorité des populations caucasiennes nord-américaines. Afin de déterminer l'effet d'une exposition prénatale aux organochlorés sur l'incidence d'infections, nous avons calculé l'association statistique entre la concentration du congénère de BPC 153 dans le sang de cordon ombilical ou le sang de la mère lors de l'accouchement et l'incidence de diagnostics d'infections respiratoires en utilisant la régression de Poisson. Les résultats tirés d'une première cohorte d'enfants suivie de 0 à 12 mois ont montré une association positive entre l'exposition aux organochlorés et l'incidence d'infections respiratoires aiguës. Cependant, l'association n'était observable que chez les enfants de moins de 6 mois et la puissance statistique n'était pas suffisante pour obtenir une association significative. Les résultats de la deuxième cohorte ont quant à eux montré une association positive statistiquement significative entre l'exposition prénatale aux organochlorés et l'incidence d'OMA et d'IVRI. Une relation dose-réponse était observable pour les OMA. Globalement, les résultats de ces études montrent qu'une proportion significative d'infections respiratoires chez les enfants

du Nunavik d'age préscolaire pourrait être due à leur exposition prénatale à des substances organochlorées.

Abstract

Inuit children from Nunavik are prenatally exposed to immunotoxic organochlorine substances (OCs) and display plasma concentrations several times higher than their counterparts from Southern Québec. This exposure tends to decrease over time but remains significantly high and could potentially alter certain immune functions. We calculated that the plasma concentration of polychlorinated biphenyls (PCB) decreased by almost 8% annually between 1994 and 2001 in Nunavik newborns. Several substances can modulate immune system functions and a wide variety of methods are available to evaluate their effects. Among them, variation in the incidence of acute respiratory infections in a given exposed population is a valid and clinically significant endpoint. To estimate the impact of exposure to OCs on the incidence of respiratory infections, we reviewed the medical charts of two cohorts of Nunavik children ($n = 199$ and $n = 354$). The calculated incidence of respiratory infections from the medical chart review during the first 5 years of life underlined a higher frequency of acute otitis media (AOM), of lower respiratory tract infections (LRTIs), and of admissions for respiratory infections, compared to most non-Inuit North-American populations. In order to determine the effect of prenatal exposure to OCs on the incidence of respiratory infections, we calculated the statistic association between plasma concentration of PCB congener 153 in umbilical cord blood or maternal blood at delivery and the incidence of diagnosed respiratory infections using Poisson regression. Results from the first cohort of children followed from 0 to 12 month of age showed a positive association between exposure to OCs and respiratory infections. However, the association was present only for children under 6 months of age and the statistical power was insufficient to reach statistical significance. Nevertheless, results from the second cohort showed a positive, statistically significant association between prenatal exposure to OCs and incidence of AOM and LRTIs. A dose-response relationship was present for AOM. Globally, these results support the hypothesis of a significant portion of respiratory infections in preschool children from Nunavik being due to prenatal exposure to OCs.

Avant-Propos

Je désire ici prendre quelques lignes pour souligner la contribution importante de plusieurs personnes tout au long de mes études doctorales. Tout d'abord, je désire remercier docteur Eric Dewailly qui m'a accueilli dans son équipe et m'a confié un projet intéressant, stimulant et déjà bien ficelé. Il a su trouver un équilibre parfait entre encadrement et latitude. J'ai aussi une dette envers docteur Gina Muckle et docteur Pierre Ayotte qui m'ont aidé, supporté et orienté tout au long de mon doctorat. Je remercie docteur Gaston De Serres d'avoir accepté la co-direction de cette thèse et d'avoir révisé et commenté les analyses et méthodes épidémiologiques que j'ai utilisées. Je tiens à souligner particulièrement la contribution de docteure Daria Pereg qui, en plus d'être une amie précieuse, a beaucoup contribué à la qualité de cette thèse par ses commentaires judicieux et ses révisions inestimables. Sa compagnie aura transformé nombres de journées ordinaires en journée fort agréables. Pour leur accompagnement, leur support et leur amitié, je ne peux passer sous silence mes collègues étudiants : Marie-Ludivine Château-Degat, Sébastien Tousignant et Michel Lucas. Finalement, je désire remercier chaleureusement Marie-Eve Ouellet qui m'a accompagné, écouté et épaulé tout au long de mon doctorat.

Les chapitres 2 à 6 ainsi que les 3 annexes de cette thèse sont des textes scientifiques que j'ai co-écrit avec d'autres auteurs. J'ai rédigé tous les articles sauf celui de l'annexe 3, que j'ai rédigé conjointement avec Cynthia Cameron. J'ai fait toutes les analyses statistiques et la plupart des interprétations de résultats. J'ai conçu le protocole et les questionnaires pour les chapitres 4 et 6, et l'annexe 3. J'ai fait l'ensemble de revue de littérature, incluant celle du chapitre 2.

Table des matières

Liste des tableaux

Liste des figures

Chapitre 1 – Introduction générale

Les organochlorés

Les organochlorés (OC) sont des composés synthétiques chlorés lipophiles. Cette famille de substance inclut, entre autres, des pesticides chlorés et leurs métabolites [dichlorodiphénylt richloroéthane (DDT), dichlorodiphenyldichloroéthylène (DDE), hexachlorocyclohexane (HCH), mirex et chlordanes], ainsi que des composés industriels chlorés [biphényles polychlorés (BPC) et hexachlorobenzène (HCB)]. Les OC les plus répandus sont le DDT, un pesticide puissant, et les BPC, dont les mélanges industriels ont servi de lubrifiant et de liquide de refroidissement dans les transformateurs électriques. Pour la période allant de 1930 à 1992, on a estimé la production mondiale de BPC à 1,2 mégatonnes (dont 31 % auraient été relâchées dans l'environnement) et la production mondiale de DDT à 2,6 mégatonnes (Barrie et al. 1992, Macdonald et al. 2000).

Le haut niveau de chloration des OC leur confère une grande stabilité. Cependant, bien que cette stabilité représente un avantage industriel indéniable, elle rend par le fait même les OC résistants à la biodégradation. Suite au constat de l'accumulation importante des OC dans l'environnement, plusieurs pays industrialisés ont décidé de bannir la production et l'utilisation des BPC et des pesticides chlorés. Au Canada, la production d'organochlorés est interdite depuis les années 1970 et seule l'utilisation de BPC dans des équipements fermés déjà existants est autorisée. Malgré ces mesures, les OC continuent à être déversés dans l'environnement suite à leur mauvais entreposage et à leur utilisation actuelle dans certains pays.

Les organochlorés présents dans l'environnement sont transportés et distribués principalement par les grands courants atmosphériques et marins. Les précipitations les réintroduisent alors dans le sol et les cours d'eau d'écosystèmes éloignés des sources de production, d'utilisation et d'entreposage. Aujourd'hui, on détecte des organochlorés dans toutes les régions du globe, incluant les écosystèmes nordiques (Barrie et al. 1992, Burkow & Kallenborn 2000, Macdonald et al. 2000, Muir et al. 1992).

L'exposition des populations inuites

La bioaccumulation dans la faune arctique

Les OC présents dans le milieu sont absorbés par la végétation et le phytoplancton. Comme ces substances sont très peu métabolisées et difficiles à excréter, elles s'accumulent dans les graisses des organismes vivants. Les poissons et autres animaux qui mangent des végétaux et du phytoplancton absorbent les organochlorés présents dans les graisses et les stockent à leur tour. Ainsi, la concentration d'organochlorés augmente avec les niveaux trophiques. Evans et al. (1991) ont montré que dans le lac Michigan, les niveaux de DDT étaient de 28,7 fois supérieurs dans le poisson que dans le plancton. Dans l'écosystème arctique, les concentrations les plus élevées d'OC se retrouvent dans les espèces prédatrices au sommet de la chaîne alimentaire (Braune et al. 1999, Dewailly et al. 1993, Muir et al. 1999, Muir et al. 1992, Skaare et al. 2000). Par exemple, à Svalbard, un archipel norvégien situé au niveau du 78^{e} parallèle, le niveau de BPC dans les tissus des goélands est de 2 à 3 fois plus élevé que celui des espèces constituant la diète du goéland. De même, les niveaux de BPC détectés dans les tissus des ours polaires sont 8 fois plus élevés que ceux des phoques. Ces concentrations de BPC sont environ 50 à 80 fois celles présents dans le lait maternel des femmes norvégiennes (Skaare et al. 2000).

L'alimentation inuite

Avant le 20^{e} siècle, la majorité des Inuit du Canada arctique chassait et pêchait pour sa subsistance. Ils vivaient en petits groupes de quelques familles et suivaient le gibier, en alternant entre les camps de pêche l'été et les igloos l'hiver. Dans la première moitié du 20^{e} siècle, les Inuit découvrirent le potentiel commercial de la traite de la fourrure et celle-ci prit de plus en plus de place dans leur mode de vie. Le trappage offrait la possibilité d'acquérir de la nourriture, des fusils de chasse et des bateaux motorisés. Pendant la même période, une diminution de la population de caribous contribua à accélérer la transition de la chasse pour la subsistance vers la traite de la fourrure. La pénurie de caribou s'accentuant, plusieurs groupes devinrent dépendants de la traite pour éviter la famine. Lors de la grande dépression et de la Deuxième Guerre Mondiale, le marché de la fourrure s'effondra complètement et plusieurs groupes inuits virent alors disparaître leur seule pos-

sibilité d'obtenir l'argent et les biens desquels ils dépendaient de plus en plus pour leur survie (Hodgins 1997).

Offrir de l'aide et des services de base aux Inuit était difficile car ceux-ci n'étaient pas sédentaires. La formation de communautés permanentes a alors été fortement encouragée par le gouvernement fédéral. En 1956, le gouvernement a commencé à construire des habitations à prix modique dans les nouvelles communautés inuites permanentes. De nouveaux villages se formaient et, bien que rudimentaires au début, les services de santé et d'éducation devenaient de plus en plus présents (Duhaime 1985, Hodgins 1997). En 50 ans, les habitants des nouvelles communautés inuites du Canada et d'ailleurs ont ainsi subi des changements culturels et sociaux aussi rapides que profonds. Par exemple, au Groenland, la proportion d'hommes pour qui la chasse est l'occupation principale est passée de 60 % en 1950 à 20 % en 1976 (Bjerregaard 1991).

Ces modifications du style de vie ont aussi profondément transformé les habitudes alimentaires des Inuit. Les sucres raffinés, la nourriture importée (porc, poulet, lait de vache) et les aliments à pauvre valeur nutritionnelle (friandises, croustilles, boissons gazeuses, etc.) se sont graduellement incorporés à leur alimentation traditionnelle (Olsen 1985). Aujourd'hui, la nourriture importée cohabite avec la nourriture traditionnelle des Inuit. Même si l'importance de cette dernière tend à diminuer, elle est encore bien présente dans les communautés. La nourriture traditionnelle représente un avantage économique, mais elle fait aussi partie intégrante des valeurs inuites. Par exemple, chez les femmes du Nunavik, l'alimentation traditionnelle fournit encore plus de 40 % de certains nutriments tels que les protéines, la vitamine D et le fer, bien que cette proportion soit plus faible chez les jeunes femmes que chez les femmes plus âgées (Blanchet et al. 2000).

L'exposition alimentaire

Puisque les OC sont emmagasinés dans les graisses et que leurs concentrations augmentent avec les niveaux trophiques, la consommation de poisson et de mammifères marins est susceptible d'augmenter l'exposition aux OC. Chez l'humain, la principale source d'exposition environnementale aux OC provient de l'alimentation. Une exposition élevée aux OC a été associée à une diète riche en poisson et en mammifères marins en Suède (Sjodin et al.

2000), au Groenland (Bjerregaard et al. 2001) et dans la région des Grands Lacs (Humphrey et al. 2000). Plusieurs études ont déjà montré que, comparativement aux habitants du sud du Québec, les habitants du Nunavik ont des concentrations moyennes d'OC dans le sang et le lait maternel significativement plus élevées (Ayotte et al. 1997, Dewailly et al. 1993, Dewailly et al. 1998a, Dewailly et al. 1989, Muckle et al. 1998, Rhainds et al. 1999).

L'exposition prénatale

Le transfert des OC de la mère vers le fœtus est connu depuis longtemps et des niveaux élevés de BPC et de pesticides sont habituellement observés dans le sang de cordon ombilical d'enfants nés de mères exposées (Ando et al. 1985, Jacobson et al. 1984, Saxena et al. 1981). Muckle et al. (1998) ont revu la littérature concernant l'exposition prénatale des enfants canadiens. Dans cette étude, les auteurs montrent que les nouveau-nés inuits du Nunavik et les nouveau-nés montagnais de la Côte-Nord sont les plus fortement exposés (moyenne géométrique de l'équivalent Aroclor 1260 de 2,0 µg/L dans le plasma du sang de cordon ombilical). Les nouveau-nés de la région de Baffin au Nunavut suivent de près avec 1,7 µg/L. Plus récemment, Muckle et al. (2001b) ont observé une exposition prénatale aux OC chez les enfants inuits du Nunavik deux à trois fois plus élevée que celle d'enfants du sud du Québec et du Massachusetts (Korrick et al. 2000, Rhainds et al. 1999). L'exposition prénatale des enfants du Nunavik était cependant moins élevée qu'au Groenland et aux Iles Faroe (Bjerregaard & Hansen 2000, Steuerwald et al. 2000).

Le BPC 153 sérique comme marqueur d'exposition

Bien que la majorité des OC soit entreposée dans les graisses, une partie de ceux-ci est présente dans le sang. Un équilibre se forme alors entre la concentration sérique et la concentration dans les tissus lipidiques. La concentration sérique est donc fonction de la concentration dans les graisses. Par contre, les lipides présents dans le sang mobilisent les OC présents dans les tissus. Ainsi, pour une même concentration tissulaire, un changement dans la concentration des lipidiques sanguins peut faire varier la concentration d'OC sériques. Pour que la concentration sérique reflète la concentration tissulaire – et donc l'exposition antérieure –, l'idéal est d'obtenir un échantillon de sang à jeun. Lorsque ce n'est pas possible, il est possible d'ajuster la concentration d'OC dans le sang en fonction des lipides sériques. Bien que cette méthode ait parfois été contestée, elle demeure une estimation valide des OC

tissulaires lorsque les lipides sériques ne sont pas un facteur de risque pour l'issue à l'étude (Schisterman 2005).

Le congénère de BPC 153 est le plus abondant dans le sang humain de personnes exposées par le biais de leur alimentation. Sa concentration est fortement associée à celle des autres congénères de BPC et à plusieurs autres pesticides chlorés. L'utilisation du BPC 153 sérique représente donc un proxy valide pour l'estimation de l'exposition aux OC dans cette population (Muckle et al. 2001b).

Les tendances temporelles de l'exposition prénatale

La plupart des OC ont été bannis dans les pays industrialisés depuis plus de 20 ans. Avec l'arrêt de la production et l'augmentation des mesures pour limiter les déversements, il serait logique d'observer une diminution lente de la contamination environnementale. En effet, la plupart des études portant sur les tendances temporelles des OC ont identifié une baisse graduelle de la contamination. Plusieurs chercheurs ont observé une diminution des OC chez différentes espèces animales depuis les années 1970 (Georgii et al. 1994, Hebert et al. 1994, Muir et al. 1999, Muir & Norstrom 2000, Roose et al. 1998, Ryckman et al. 1994, Schmitt et al. 1999). Chez l'humain exposé environnementalement, les niveaux de BPC dans le lait maternel ont diminué depuis les 15 dernières années en Allemagne (Schade & Heinzow 1998) et en Suède (Noren 1993, Noren & Meironyte 2000). Des tendances descendantes dans le sang ou les graisses ont aussi été observées au Michigan (He et al. 2001), au Mexique (Waliszewski et al. 1998), sur la Côte-Nord du St-Laurent (Dallaire et al. 2002) et en Angleterre (Harris et al. 1999). Une exception notable à cette règle est cependant observée avec les biphenyléthers bromés, dont la concentration augmente dans le lait maternel des femmes du Nunavik et d'autres régions du globe (Pereg et al. 2003). L'évaluation des tendances temporelles de l'exposition prénatale aux OC chez les enfants du Nunavik fait l'objet d'un chapitre dans cette thèse (chapitre 3).

Les effets sur la santé

Les BPC et leurs sous-produits

Une imposante revue de littérature de Stephen H. Safe (1994) énumère les nombreux effets toxiques des BPC commerciaux sur des modèles animaux. Plus de 150 études ont été effectuées pour mieux comprendre et décrire la toxicité des BPC. Des effets néfastes sur la reproduction, la croissance, le système immunitaire, le système hépatique, le système endocrinien (particulièrement sur les hormones thyroïdiennes), le système nerveux et la peau ont été documentés.

Malheureusement, les effets d'une exposition aux BPC chez l'humain sont moins bien connus. La contamination d'huile de riz par des BPC au Japon en 1968 (maladie de Yusho) et à Taiwan en 1979 (maladie de Yu-Cheng) ont permis d'obtenir de l'information précieuse sur l'effet d'une forte exposition aux BPC et à leurs produits de dégradation chez l'humain (Aoki 2001). Depuis, des cohortes de sujets exposés soit par le biais de leur alimentation, soit parce qu'ils habitent à proximité d'un site contaminé, ont été mises sur pied afin de mieux décrire et comprendre les effets de ces substances chez l'humain.

Les effets neurologiques

Les premiers effets neurologiques des BPC (et de leurs produits de dégradation thermique) ont été observés chez des sujets exposés accidentellement (Yusho et Yu-Cheng). Les enfants nés de mères exposées avaient de moins bonnes fonctions intellectuelles que des enfants nés de mères non exposées (Chen et al. 1992, Yu et al. 1991). Suite à ces observations, des chercheurs se sont penchés sur les effets neurodéveloppementaux d'une exposition environnementale. Une étude réalisée en Caroline du Nord a montré que des enfants exposés aux BPC avaient un tonus diminué et de moins bons réflexes à la naissance que des enfants moins exposés. Les enfants exposés avaient un développement moteur plus lent et un déficit de la mémoire visuelle et de la mémoire à court terme (Rogan & Gladen 1992). Dans une étude impliquant des enfants nés de mères qui consomment beaucoup de poissons du lac Michigan, l'équipe du docteur Jacobson a mis en évidence une relation inverse entre l'exposition prénatale aux BPC et la reconnaissance visuelle du nourrisson (Jacobson et al. 1992, Jacobson et al. 1985), relation qui a été confirmée par la suite (Darvill et al. 2000).

Des effets semblables ont été mis en lumière par Huisman et al. (1995). L'équipe hollandaise a découvert qu'il existait une relation inverse entre la teneur de BPC dans le lait maternel et un développement neurologique optimal de l'enfant. Une relation entre les BPC et une fonction intellectuelle diminuée a aussi été identifiée (Jacobson & Jacobson 1996, Patandin et al. 1999).

Les effets endocriniens

Bien que beaucoup de chercheurs se soient penchés sur la question depuis les 10 dernières années, la relation entre l'exposition environnementale aux BPC et les niveaux néonataux d'hormones thyroïdiennes reste controversée (Vulsma 2000). Dans une étude de Longnecker et al. (2000), seule une faible relation entre l'exposition prénatale aux BPC et les niveaux de TSH dans le sang de cordon ombilical a pu être détectée. En Hollande, des chercheurs ont aussi pu détecter une relation entre les niveaux de thyroxine totale du nouveau-né et la concentration de BPC dans le lait maternel (Pluim et al. 1993, Pluim et al. 1992). Des résultats similaires ont été publiés par une autre équipe hollandaise une année plus tard (Koopman-Esseboom et al. 1994). Ces effets des BPC sur les hormones thyroïdiennes sont cependant faibles et les concentrations d'hormones sont toujours demeurées à l'intérieur des standards cliniques.

Les effets sur le système immunitaire chez l'humain (exposition accidentelle)

Les premières évidences d'un effet immunotoxique des BPC et de leurs produits de dégradation chez l'humain proviennent de l'évaluation de sujets ayant consommé du riz contaminé lors de l'accident Yu-Cheng. Chang et al. (1981), ont observé une diminution des concentrations d'immunoglobulines A (IgA) et d'immunoglobulines M (IgM) et une perturbation des populations de lymphocytes T chez 30 sujets exposés, comparativement à 23 sujets sains. L'année suivante, le même groupe a identifié une suppression de l'immunité cellulaire (hypersensibilité retardée) chez les mêmes sujets (Chang et al. 1982). Lü et Wu (1985) ont montré que la plupart des fonctions immunitaires de ces sujets étaient revenues à la normale trois ans plus tard.

En 1971, de la boue contaminée à la TCDD a été mélangée à de l'huile puis étendue sur une route de terre au Missouri. Hoffman et al. (1986) ont examiné 154 sujets exposés et les

ont comparés à 155 sujets contrôles. Les auteurs ont identifié une augmentation de la fréquence d'anergie et ont observé des anomalies non-significatives dans les populations et fonctions de lymphocytes T. En 1976, une zone résidentielle a été contaminée au TCDD suite à un accident industriel à Seveso, en Italie. Vingt ans après l'accident, Baccarelli et al. (2002) ont évalué certaines fonctions immunitaires chez 62 sujets exposés et 58 témoins. Les auteurs ont identifié une association négative entre les concentrations de TCDD et les niveaux d'IgG, sans que les autres immunoglobulines ou protéines du complément ne soient affectées.

Les effets sur le système immunitaire chez l'humain (exposition environnementale)

Dans les études des effets immunotoxiques d'exposition environnementale aux contaminants, il est souvent difficile d'attribuer les effets observés à une molécule en particulier puisque les sujets sont généralement exposés à un mélange complexe de substances. Ainsi, les concentrations de BPC sont souvent corrélées à d'autres OC, tels que le DDT et le DDE. Bien que la plupart des études portent sur les BPC, il est impossible de savoir si les effets observés sont dus aux BPC, à d'autres substances, ou à une combinaison des effets des différentes substances dans le mélange.

Svensson et al. (1994) ont évalué le système immunitaire de 23 hommes qui consommaient fréquemment du poisson, et l'ont comparé à celui de 20 hommes qui consommaient très peu de poisson. Ils ont observé une diminution de la population totale et relative de cellules dites tueuses (*natural killers* ou cellules NK), mais n'ont pas observé d'effets sur les autres populations lymphocytaires, ou sur les concentrations d'immunoglobulines.

Dans une cohorte de 207 enfants hollandais sains, des chercheurs ont identifié une relation positive entre l'exposition prénatale aux BPC et le nombre de lymphocytes T TcR$\gamma\delta^+$ à la naissance, et le nombre de lymphocytes T totaux, $CD8^+$, TcR$\alpha\beta^+$, et TcR$\gamma\delta^+$ à 18 mois (Weisglas-Kuperus et al. 1995). Une relation négative entre l'exposition post-natale et les comptes de monocytes et de granulocytes a aussi été observée. Chez les mêmes enfants, à 42 mois, les auteurs ont aussi observé une relation positive entre l'exposition prénatale et le nombre total de lymphocytes, le nombre total de lymphocytes T, et le nombre de lymphocytes T cytotoxiques ($CD3CD8^+$), mémoires ($CD4^+CD45RO^+$) et activées ($CD3^+$HLA-

DR^+) (Weisglas-Kuperus et al. 2000). Une association négative entre la réponse vaccinale et l'exposition prénatale a aussi été identifiée dans la même étude.

Au Japon, Nagayama et al. (1998b) ont identifié une relation positive entre une estimation de l'exposition aux BPC (et leurs sous-produits) par le biais du lait maternel et le ratio $CD4^+/CD8^+$ chez 36 nouveau-nés. En Belgique, Van Den Heuvel *et al.* (2002) ont montré que chez 200 adolescents belges, l'exposition aux BPC était associée négativement au pourcentage d'éosinophiles, au pourcentage de cellules NK et aux niveaux spécifiques d'IgE contre 3 allergènes communs. L'exposition aux BPC était aussi associée positivement au niveau d'IgA.

Finalement, Belles-Isles et al. (2002) ont comparé certains paramètres immunitaires dans des échantillons de sang de cordon ombilical prélevés chez 48 nouveau-nés de la Côte-Nord et 60 nouveau-nés témoins de Sept-Iles. Les familles des nouveau-nés de la Côte-Nord avaient des niveaux de BPC 2 à 3 fois plus élevés que ceux de Sept-Iles. Dans cette étude, les BPC étaient négativement associés à la proportion de lymphocytes T $CD4^+CD45RA^+$ et à l'expansion clonale des lymphocytes T. Une autre étude chez la même population a pu mettre en évidence une relation inverse entre l'exposition prénatale aux OC et la production de cytokines *in-vitro* suite à une stimulation mitogénique des leucocytes (Bilrha et al. 2003). Dans cette étude, une corrélation négative a été observée entre la production de facteur de nécrose tumorale (*tumor necrosis factor* ou TNF-α) *in-vitro* et la concentration de OC dans le sang.

Il est difficile de comparer ces études entre elles. Les sources et les mesures d'exposition varient, différentes fonctions immunitaires ont été mesurées et les sujets sont d'âge différents. De plus, plusieurs autres fonctions du système immunitaire étaient mesurées sans montrer d'association significative. À la lumière de ces résultats, on ne retrouve pas de constance dans les fonctions immunitaires affectées pas les OC. Ce sont les diminutions de concentrations d'immunoglobulines et les réductions des proportions de cellules NK qui sont les effets les plus souvent rapportés. D'autres études sont encore nécessaires pour bien identifier les perturbations du système immunitaire suite à une exposition accidentelle ou environnementale aux OC.

Les mécanismes des effets sur le système immunitaire

Les mécanismes précis des effets immunotoxiques des BPC sont encore mal compris. La piste la plus explorée demeure la liaison au récepteur des hydrocarbure aromatique (Ah), particulièrement par les congénères de BPC qui adoptent une conformation planaire. Cette conformation, qui est due à la chloration du noyau en position 3, 3', 4 et 4', ressemble à celle de la 2,3,7,8-tetrachlorodibenzo-*p*-dioxine (TCDD). La TCDD, ou dioxine, est le meilleur ligand connu du récepteur Ah. Chez le rongeur, la suppression de la réponse immune par des congénères de BPC est proportionnelle à l'affinité pour le récepteur Ah (Holsapple et al. 1991, Kerkvliet 1995). De même, les souris qui n'expriment pas le récepteur Ah sont moins susceptibles aux effets immunotoxiques de la TCDD, tout en demeurant capables de monter une réponse immune normale (Vorderstrasse et al. 2001). Le récepteur semble donc en partie responsable de l'effet immunotoxique, mais il n'est pas indispensable pour le développement de la réponse immune. Ceci étant dit, très peu de choses sont connues concernant les conséquences cellulaires de la liaison d'un ligand au récepteur Ah (Kerkvliet 2002). D'autres effets immunotoxiques de la TCDD, cette fois indépendants du récepteur Ah, ont aussi été observés. Plusieurs hypothèses ont été émises (perturbation hormonale, surproduction d'acide arachidonique), mais aucune n'a été suffisamment appuyée (Kerkvliet 2002).

Bien que les mécanismes soient encore peu caractérisés et compris, les effets immunotoxiques des composés semblables à la dioxine ont été plusieurs fois observés. Ainsi, la myélosuppression, l'atrophie du thymus, l'immunosuppression, la diminution des concentrations d'IgA et d'IgM, l'inhibition du complément et l'altération des sous-populations lymphocytaires ont toutes été observées chez le rongeur et le singe (Esser & Welzel 1993, Holsapple et al. 1984, Neubert et al. 1992, Thomas & Hinsdill 1979, Tryphonas et al. 1991a, Tryphonas et al. 1991b, White et al. 1986).

Le DDT et le DDE

Longnecker et al. (1997) ont revu la littérature concernant les effets du DDT et des composés apparentés et ont conclu qu'il existait peu de preuves d'effets toxiques d'une exposition environnementale au DDT. Seule la durée de l'allaitement semble être diminuée suite à une exposition au DDT (Rogan et al. 1987). Des études récentes montrent qu'une exposition au

DDT, par le biais du lait maternel, pourrait modifier la croissance à la puberté (Gladen et al. 2000) et affecter l'équilibre des hormones thyroïdiennes des nourrissons (Nagayama et al. 1998a). Récemment, Daniel et al. (2002) ont évalué plusieurs fonctions immunitaires chez 49 personnes exposées aux insecticides en milieu agricole dans l'ex-Allemagne de l'Est. Les niveaux plasmatiques de DDT et de DDE étaient corrélés aux niveaux d'interleukines (IL)-2 et d'IL-4. Cependant, plusieurs autres fonctions immunitaires ne montraient pas d'association avec les niveaux de DDT/DDE et il est probable que les associations observées soient dues au hasard. Dans l'ensemble de ces études, les concentrations de DDT et de DDE sont souvent corrélées à celles d'autres OC. Il est donc difficile de parler d'un effet – ou d'une absence d'effet – spécifique au DDT et au DDE.

Le HCB et les chlordanes

Les effets du HCB chez l'humain sont peu connus. Les principaux effets sur la santé sont la porphyrie (Daniell et al. 1997) et le cancer (Cabral et al. 1977). Ce composé aurait aussi certaines propriétés immunotoxiques (Michielsen et al. 1999). Une intoxication aiguë aux chlordanes affecte le système nerveux (Grutsch & Khasawinah 1991). Par contre, il n'existe pas d'étude claire quant aux effets d'une exposition chronique. Les chlordanes favoriseraient la croissance de tumeurs (Dich et al. 1997) et ont été associées quelques fois à une faible augmentation de l'incidence de certains cancers (Safety 1984).

Les OC et la susceptibilité aux infections chez les enfants

Ultimement, la question d'intérêt en santé publique est de savoir si les effets biologiques rapportés se soldent par une diminution de la résistance aux infections. Le système immunitaire semble plus vulnérable aux effets des OC dans la période périnatale (Badesha et al. 1995, Barnett et al. 1987). Chez les modèles animaux, il a été observé qu'une exposition aux OC ne causant pas d'effet marqué chez la mère pouvait avoir des effets néfastes, quelques fois permanents, sur le système immunitaires des nouveau-nés (Badesha et al. 1995, Gehrs & Smialowicz 1999, Luebke 2002, Tryphonas et al. 1991b). Ainsi, la majorité des études portant sur la susceptibilité aux infections a été réalisée avec des enfants exposés dans la période périnatale.

L'effet d'une exposition environnementale aux OC sur la susceptibilité aux infections chez l'humain demeure controversé. Peu d'études ont été réalisées et les résultats divergent. À notre connaissance, la première étude sur la question a été effectuée dans la région des Grands Lacs. La cohorte mères/enfants du Wisconsin (*The Wisconsin Maternal/Infant Cohort*) a été élaborée pour évaluer l'effet sur la santé des enfants suite à une exposition aux BPC par le biais de la consommation de poisson. Les auteurs ont observé que la consommation de poisson pendant la grossesse était associée positivement avec l'occurrence de symptômes de rhume, de douleurs aux oreilles et de grippe (Smith 1984). Entre 1978 et 1982, Rogan et al. (1987) ont suivi environ 900 familles en Caroline du Nord (États-Unis). Les auteurs ont révisé les dossiers médicaux des enfants et n'ont pas trouvé d'évidence d'un effet néfaste d'une exposition aux BPC ou au DDE dans la première année de vie. En Hollande, l'équipe du docteur Weisglas-Kuperus n'a pas observé d'association entre l'exposition prénatale aux BPC et le nombre d'épisodes de rhinite, de bronchite, d'amygdalite et d'otite pendant les 18 premiers mois de vie (Weisglas-Kuperus et al. 1995). Cependant, le même groupe d'enfants a été revu et les concentrations de BPC dans le sang à 42 mois étaient cette fois associées positivement à la prévalence d'otites récurrentes et de varicelle (Weisglas-Kuperus et al. 2000). Récemment, les parents de 167 enfants de cette cohorte ont répondu à un questionnaire sur la santé de leur enfants qui sont maintenant d'âge scolaire. Les auteurs ont montré que l'exposition post-natale était toujours associée au risque d'otites moyennes récurrentes, et que l'exposition prénatale était associée à une réduction des symptômes d'asthme (Weisglas-Kuperus et al. 2004). Karmaus et al. (2001) ont aussi observé un risque augmenté d'otites chroniques, mais l'association était seulement présente avec un modèle combinant l'exposition aux BPC et au DDE. Une étude réalisée par notre groupe de recherche a montré que les enfants inuits plus fortement exposés avaient un risque augmenté d'otites moyennes aiguës pendant la première année de vie (3^{e} tertile d'exposition comparé au premier tertile). La relation n'était significative qu'avec l'exposition au DDE et au HCB, mais était positive aussi pour les BPC, le dieldrine et le mirex (Dewailly et al. 2000).

Encore une fois, ces études ont des devis très différents et sont difficiles à comparer entre elles. Qui plus est, très peu d'études ont évalué simultanément la fréquence d'infection et les paramètres du système immunitaire. Il est donc difficile de savoir quels mécanismes –

ou quelles fonctions du système immunitaire – pourraient être responsables des effets observés.

Les infections chez les enfants inuits

L'incidence de maladies infectieuses chez les enfants inuits est systématiquement plus élevée que celle des enfants caucasiens vivant plus au sud (Banerji et al. 2001, Butler et al. 1999, Holman et al. 2001, Koch et al. 2002, Wainwright 1996). Les enfants inuits du Canada, du Groenland et de l'Alaska sont ceux qui ont la prévalence d'otites la plus élevée au monde (Bluestone 1998). En Alaska, il a été montré que, comparativement aux enfants caucasiens, les enfants autochtones et inuits ont des taux plus élevés d'infections à pneumocoque (Davidson et al. 1994, Wainwright 1996), d'hospitalisations pour infections au virus respiratoire syncytial (Karron et al. 1999, Lowther et al. 2000, Wainwright 1996) et d'hospitalisations pour infections respiratoires (Holman et al. 2001). Des taux d'incidence et d'hospitalisation élevés pour les infections des voies respiratoires inférieures (bronchites, bronchiolites et pneumonies) ont aussi été observés dans la région de Baffin (Banerji 2001, Banerji et al. 2001) et au Groenland (Koch et al. 2002).

À l'exception des otites, l'incidence de maladies infectieuses a été peu étudiée au Nunavik. Une étude de Dewailly et al. (2000) sur l'effet des contaminants sur l'incidence d'infection a montré que le risque d'avoir au moins un épisode d'infection respiratoire avant l'âge de un an y était de 59,3 %. Chez les enfants de moins de 3 mois, 18,5 % avaient eu un épisode de bronchite ou de bronchiolite, et 6,2 % avaient eu un épisode de pneumonie. Il a aussi été observé dans le passé que l'incidence de méningite et d'infections respiratoires était élevée chez les enfants du Nunavik (Duval & Thérien 1982, Proulx 1988).

Les otites

Au Nunavik, ce sont les otites qui ont retenu l'attention des autorités de santé publique. En 1987, un projet pilote évaluant la prévalence d'otites et de déficit de l'ouïe a été réalisée à Kuujjaraapik, sur la côte est de la Baie d'Hudson, dans le nord du Québec. Les chercheurs ont comparé la prévalence d'otites entre des enfants cris et inuits. La prévalence d'otites chroniques était de 8,2 % chez les Inuit et de 1,4 % chez les Cris. La prévalence de problèmes de l'ouï et de cicatrices du tympan étaient aussi plus élevées chez les Inuit (Baxter et

al. 1986, Julien et al. 1987). Le projet a été étendu à 11 autres communautés inuites du nord du Québec. Sur les 800 enfants inuits participant à l'étude, 85 % avaient des anomalies de l'oreille (perforation du tympan, cicatrices sur le tympan et/ou suppuration active) et 40 % avaient un déficit de l'audition (Thérien 1988).

Dans la communauté d'Inukjuaq au Nunavik (Baie d'Hudson), la prévalence d'otite chronique chez 122 enfants d'âge préscolaire était de 9,4 % en 1987, et de 10,8 % en 1997. Les facteurs de risque associés étaient le nombre d'habitant par pièce, le nombre de frères et sœurs ayant des problèmes d'otites, l'âge à la première visite au centre de santé et le type de lait en bouteille donné aux enfants (Bruneau et al. 2001). Une étude de Dewailly et al. (2000) a aussi montré un risque élevé d'otite dans la première année de vie au Nunavik (80,5 % des enfants ont eu au moins un épisode et 9 % ont eu 5 épisodes et plus). Des risques semblables ont été observés chez les enfants inuits du Groenland (Homoe et al. 1999).

Hypothèses et objectifs

Le Nunavik est une vaste région couvrant toute la partie nord du Québec. Environ 9 600 Inuit y vivent, répartis dans 14 communautés (Ministère des Travaux publics et des Services gouvernementaux du Canada 2003). L'alimentation traditionnelle, composée d'animaux et de poisson de hauts niveaux trophiques, expose les Inuit à des concentrations élevées d'OC. Le passage des OC à travers le placenta et dans le lait maternel contribue à l'exposition prénatale et postnatale des enfants inuits.

L'incidence d'infections est élevée chez les enfants du Nunavik. Les otites fréquentes y représentent un défi important pour les autorités de santé publique depuis plus de 30 ans. Les enfants du Nunavik, exposés à des substances immunotoxiques avant la naissance et affectée par un taux élevé d'infections aiguës, forment une population idéale pour l'étude des effets immunotoxiques d'une exposition prénatale aux OC chez l'humain.

Dans un premier temps, j'ai revu la littérature sur les méthodes de recherche en immunotoxicologie humaine (chapitre 2). L'objectif principal était d'identifier quelles sont les approches possibles pour identifier un effet immunotoxique dans une population humaine exposée à un agent immunotoxique. Les principes épidémiologiques de base, les facteurs à

considérer, ainsi que les principaux marqueurs biologiques utiles en immunotoxicologie humaine y sont décrits. J'y aborde aussi sommairement l'évaluation des effets immunotoxiques sur la santé, principalement sur la susceptibilité aux infections.

Dans le contexte d'une transformation des habitudes alimentaires des Inuit et d'une diminution de la contamination environnementale suite au bannissement de la production des OC persistants, nous avons émis l'hypothèse d'une tendance temporelle descendante de l'exposition environnementale des mères en âge de procréer, et donc de l'exposition prénatale de leurs enfants. Le troisième chapitre aborde cette question. L'objectif de cette étude était d'identifier une tendance temporelle dans le sang de cordon ombilical d'enfants du Nunavik nés entre 1994 et 2001. Une étude semblable a aussi été réalisée chez les nouveau-nés de la Basse-Côte-Nord (annexe 1).

Concernant les effets immunotoxiques des OC sur la santé des enfants, notre hypothèse de base était qu'une exposition prénatale augmente l'incidence et la sévérité des infections aiguës chez les enfants. Plus précisément, nous avons postulé que :

- le nombre de consultations médicales menant à un diagnostic d'infection aiguë dans un intervalle de temps donné (l'incidence de consultation) est associé à la concentration d'OC dans le sang de cordon ombilical, ou dans le sang de la mère pendant la grossesse ;
- l'incidence d'hospitalisation pour une infection aiguë est associée à la concentration d'OC dans le sang de cordon ombilical, ou dans le sang de la mère pendant la grossesse.

Pour vérifier ces hypothèses, nous avons entrepris d'estimer l'incidence d'infection en révisant les dossiers médicaux des enfants, afin de consigner chaque consultation médicale menant à un diagnostic d'infection aiguë. Pour ce faire, nous avons étudié deux cohortes d'enfants du Nunavik. La première cohorte était nichée dans une étude longitudinale sur l'effet des OC et des métaux lourds sur le développement de l'enfant, conduite par Dr Gina Muckle (Muckle et al. 2001b). Les objectifs de notre étude étaient d'évaluer l'association entre la concentration d'OC dans le sang maternel et l'incidence d'infection dans les 12 premiers mois de vie (chapitre 5). Dans cette étude, nous avons aussi vérifié l'effet d'une exposition post natale (OC dans le sang des enfants à 7 mois).

La deuxième cohorte était constituée d'enfants inuits du Nunavik ayant participé à une étude sur les contaminants dans le sang de cordon ombilical entre 1993 et 1996 (Dewailly et al. 1998a). L'objectif principal de cette étude était d'évaluer l'association entre les concentrations d'OC dans le sang de cordon ombilical et l'incidence d'infection et d'hospitalisation pendant les cinq premières années de vie (chapitre 6).

Finalement, grâce à cette dernière étude, l'information amassée par le biais de la revue de dossiers médicaux d'enfants répartis dans plusieurs villages nous a permis de dresser un portrait des consultations et hospitalisations pour infections aiguës au Nunavik. L'objectif était de mieux documenter l'incidence d'infections et d'hospitalisations chez une population connue pour son incidence élevée de maladies infectieuses(chapitre 4).

Accessoirement, les données accumulées au cours des différentes études présentées dans cette thèse ont permis d'étudier la déficience en vitamine A à la naissance ainsi que son effet sur l'incidence d'infections aiguës au Nunavik. Deux études sur le sujet sont présentées aux annexes 2 et 3.

Chapitre 2 – Méthodes de recherche en immunotoxicologie humaine

Dallaire F., Dewailly E., Ayotte P., 2005. Approaches to immunotoxicology in human population studies, dans *Investigative Immunotoxicology*, edité par Tryphonas H., Fournier M., Blakley B.R., Smits J.E.G., Brousseau P., Boca Raton: Taylor & Francis, pages 247-65.

Résumé

Les études immunotoxicologiques chez l'humain posent plusieurs défis épidémiologiques. Les devis d'étude expérimentale sont rarement possibles et les chercheurs sont forcés de se tourner vers l'identification de sujets exposés et témoins. La validité des études repose sur le choix d'un devis d'étude adéquat, mais aussi sur la compréhension des avantages et inconvénients du devis choisi. Une bonne identification des populations exposées et non-exposées, une hypothèse élaborée avec attention et des mesures valables d'exposition, d'issues et de facteurs confondants sont cruciales. Plusieurs marqueurs biologiques sont disponibles pour mesurer l'immunotoxicité, mais ils ne sont pas tous applicables chez l'humain. Les méthodes les plus reconnues sont le phénotypage des lymphocytes, l'évaluation de l'activité des cellules NK, les tests cutanés d'hypersensibilité retardée et la réponse vaccinale. Certaines batteries des tests prédétermiées existent, mais elles ont rarement été utilisées intégralement. L'inclusion d'issues cliniques, telle que l'incidence d'infection, est recommandée pour permettre une meilleure compréhension de la relation entre les marqueurs biologiques, le système immunitaire et la santé des populations humaines exposées.

Summary

Human immunotoxicology poses several epidemiologic challenges. Experimental designs are rarely feasible and the investigator is forced to rely on careful identification of exposed and control subjects. Valid studies depend on the choice of an adequate study design with an appropriate understanding of the advantages and drawbacks of the selected design. A proper identification and selection of the exposed and unexposed populations, a carefully elaborated hypothesis, and high-quality measurements of the exposures, endpoints, and

confounding factors, are also crucial. Many biological endpoints are available to assess immunotoxicity, but not all can be applied to human studies. The most acknowledged methods are the phenotyping of lymphocytes, the evaluation of NK-cell activity, skin tests for delayed-type hypersensitivity, and response to vaccination. Complete testing schemes already exist, but they have rarely been used. The inclusion of acute clinical endpoints, such as the incidence of infections, is warranted to better understand the relations between biological markers, the immune system, and the health status in human populations.

Introduction

The assessment of immunotoxicity in humans involves several epidemiological and ethical challenges. Experimental designs are rarely feasible and investigators have to rely on the identification of exposed and unexposed populations to conduct their studies. These populations differ in regards to disease prevalence, access to medical treatment, and to genetic and environmental factors. Furthermore, the function of the immune system has to be investigated by strictly non-invasive methods that are not always easy to standardize. In this chapter, we discuss epidemiological designs, factors to consider, and some useful biological and clinical endpoints relevant to human immunotoxicology. Whenever possible, we also provide examples of the methods discussed by referring the reader to published studies on the topic.

Epidemiological considerations

Human immunotoxicology studies are subject to the same rules, challenges, methods and problems as any other epidemiology study. It is well beyond the scope of this work to discuss the many subtleties of epidemiology and population studies. For a more detailed presentation, the reader is referred to the many excellent publications on epidemiology including that of Rothman and Greenland (1998). For the purpose of this discussion, only those epidemiologic principles most relevant to immunotoxicology will be presented.

Study design

For obvious ethical reasons, most studies in human immunotoxicology are non-experimental, that is, the exposure to the xenobiotic under study is not assigned by the investigator for the purpose of a study; it is the participants that, willingly or not, have

exposed themselves to the substance. In non-experimental human studies, investigators have to identify two groups of participants, one exposed and the other unexposed (or less exposed), that are in similar situations. These situations should be comparable enough so that the differences between the two groups will not influence the disease under study more than the exposure itself. In this section, we will briefly explore three non-experimental study designs: a cohort study, a case-control study, and a cross-sectional study. The reader should be aware of the existence of other designs, as well as of the many variations within the three designs mentioned (Rothman & Greenland 1998).

Cohort studies

To design a cohort study in immunotoxicology, the investigator has to define various groups of participants differing by their exposure to a xenobiotic. The most important aspect of a classical cohort study is that the recruited participants must be free of the disease under investigation at the beginning of the study. A cohort study will follow the participants over time and compare the incidence rates between exposure groups, i.e. the rates of negative participants turning positive for a given endpoint. This design is the most straightforward, but is also the most expensive and time consuming. In classical cohort studies, the endpoint is usually the onset of a disease and the analyses are conducted to determine the differences of incidence of this disease between the exposure groups. Nevertheless, cohort studies can also be used with continuous biological endpoints, such as the ones often used in immunotoxicology. In this case, the cohort design has the enormous advantage of allowing the investigators to follow the participants in order to record the variations of a given endpoint during the study time-course. Finally, it is often possible to incorporate other types of design within a cohort study setting. Such concepts are better understood when specific examples are considered.

Rogan et al. (1987) used a cohort design to evaluate the effects of polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethylene (DDE) on growth, morbidity, and duration of breast-feeding in children. The neonates were grouped according to exposure levels and the incidence of illnesses was recorded during the first year of life. Considering that the recorded illnesses were ‘new’ illnesses, i.e. illnesses not present at recruitment, the design would correspond to that of a cohort study. Similarly, Weisglas-Kuperus et al.

(2000) investigated the effects of PCBs and dioxins on infections in children. Again, only healthy newborns were recruited and 'new' infections were considered. In the latter study, the authors also evaluated T-cell markers. In this case, the design did not technically correspond to a cohort study since T-cell markers were not measured at birth. When only one measurement is made, one evaluates the relation between exposure and the endpoint at one specific time instead of evaluating the effect of exposure on the 'progression' of this endpoint. This corresponds to a cross-sectional design since the investigator could not ascertain that the difference observed at the end of the study was not present at the beginning (see section on cross-sectional design for further discussion on this topic).

When one wants to benefit from the advantage of a cohort design using biologic endpoints, which is to evaluate the variation of the endpoint during the follow-up, one has to measure the endpoint at the beginning (baseline) and at the end of the follow-up. This allows for the calculation of the difference between the level at the end of the follow-up and the baseline level. This strategy will help dealing with inter-individual variations of the baseline level of the given endpoint. Statistically, this would be achieved in multiple regression by inserting a variable representing the difference between the two measurements into the regression model as the main dependent variable. More sophisticated strategies exist, such as time series and hazard functions, when more than two measurements are made.

Case-control studies

Case-control studies are generally used when the disease under investigation is rare so that it would be impractical or too expensive to recruit healthy subjects and follow them until a sufficient number is diagnosed with the disease (as in a cohort study). It should be noted, however, that the rarity of the disease is in most cases not an essential criterion for a case-control study to be valid (Rothman & Greenland 1998). To conduct a case-control study, one has to identify cases, preferably subjects newly diagnosed with the disease, in a source population. One will also have to recruit controls, i.e. participants who are free of the disease, within the same source population. The key aspect in case-control studies is that the participants must be selected independently from their exposure status. The comparison of the proportions of exposed and unexposed participants between the cases and the

controls will yield an estimate of the relative risk (odd ratio) between the exposed and unexposed subjects.

In the field of immunotoxicology, case-control studies should be used to investigate potential associations between the exposure to xenobiotics and the development of chronic diseases such as cancer or systemic lupus erythematosus. Hardell and Ericsson (1999) used the case-control design to evaluate the association between non-Hodgkin lymphoma and exposure to pesticides. The authors identified 404 Swedish males diagnosed with non-Hodgkin lymphoma, and 741 controls. Exposure to pesticides was estimated by the use of a standardized questionnaire and a telephone interview. By doing this, they found an association between the risk of non-Hodgkin lymphomas and previous exposure to herbicides, insecticides, and fungicides (odd ratios of 1.6, 1.2, and 3.7, respectively).

The case-control design also allows for the comparison of continuous variables between the cases and the controls. For example, one could assess the level of pesticides in the plasma of the participants and compare the mean concentrations between the cases and the controls. This can give an insight on the difference between both groups without having to create categories from a continuous variable (for instance grouping the participants by quartiles of exposure). This can be done for every assessed variable. However, to remain valid, the recruitment must be done independently from such variables, i.e. the factors shall not be a selection criterion in neither cases nor control (Rothman & Greenland 1998).

Cross-sectional studies

A cross-sectional study design is defined as a study in which participants are recruited at one time point, irrespective of their status regarding the particular endpoint to be investigated**.** In this design, the objective is to describe the status of a population at one point in time (a 'cross-section' in time). The exposure status and the endpoint are usually investigated simultaneously.

The main limitation of cross-sectional study designs is that only one measurement of the endpoint is performed, without any follow-up. If the endpoint is a disease, the information obtained by the investigator is a disease prevalence, i.e. the proportion of participants having the disease at one point in time. When disease prevalence is used in epidemiology,

the investigator faces the problem of length bias (Rothman & Greenland 1998). Disease prevalence is a function of both the incidence and the duration of the disease (the longer the disease is present in an individual, the higher is the probability of recruiting a participant having the disease at one point in time). Consequently, cross-sectional designs do not allow for the discrimination between an effect on the incidence of an event and an effect on the duration of the same event. Furthermore, the valuable information on the variation or on the progression of an endpoint during the follow-up is impossible to obtain. The strategy where a participant serves as its own control, such as in cohort designs, is, therefore, not applicable, and inter-individual variations will affect the results in a greater fashion.

Despite these drawbacks, cross-sectional studies are often used for practical and financial reasons, and a well-executed cross-sectional study may yield valuable and valid information. Svensson et al. (1994) used this design to evaluate the association between fish consumption and several immune parameters in Swedish adult males. These investigators recruited fish eaters and non-fish eaters as a surrogate of organochlorines (OCs) exposure and evaluated, among other endpoints, T-cells markers. They found a lower proportion of natural killer (NK) cells in fish eaters as compared to non-fish eaters. This result is subject to the limitations mentioned above but is still very useful. The insight on the potential effect of OCs on NK cells would most likely have been much more expensive to gain from a cohort study.

Confounding

Reducing the random error in a study, and therefore increasing the precision, is important to obtain statistically significant results. Failing to do so, however, will not bias the study results. A study with high random error will most likely produce non-significant results but will still be valid. It is the systematic error – or the differential error – that will bias the results. It would be too exhaustive to discuss all of the possible biases and systematic errors. However, confounding is a key aspect in the validity of any epidemiological study, and we feel that this aspect needs to be addressed in some detail.

There are three essential characteristics for a factor to confound an association. Firstly, it must affect the endpoint under study. Secondly, it must be associated with the exposure

and, thirdly, the factor must not be an intermediate step in the causal chain of the postulated mechanism of effect. Of all the factors that could affect the immune system, those which fulfill these three criteria could potentially become confounders.

Let us suppose that an investigator wants to study the effect of occupational exposure to dioxins on the antibody response to vaccination. If the participants exposed to dioxins are also exposed to excessive stress due to their work environment, then stress could possibly confound the association. In this set up, stress is known to affect antibody response and is also associated with the exposure. Furthermore, stress is not an intermediary step in the postulated mechanism of dioxin toxicity, that is, the hypothesis does not state that the effect of dioxin on humoral response is the result of stress. This means that a potential association between dioxin exposure and response to vaccination could partly be due to the effect of stress, therefore introducing a bias in the results.

In the example above, the 'stress' factor displays the three essential characteristics to become a confounder. It does not mean that it will necessarily confound the association. It is a common misconception that any factor associated with the endpoint and the exposure must be controlled for in the analysis. Observing a significant association between a factor and the endpoint (a p-value < 0.05) is not a criterion for the inclusion of this factor in a statistical model when a causal hypothesis is investigated. One must ensure that the factor could be a true confounder. Firstly, the factor must influence the endpoint under study, i.e. be a risk factor for the endpoint; it is not enough to be merely statistically associated with the endpoint. Secondly, the inclusion of a variable representing the potential confounder in a statistical model must affect the association between the exposure and the endpoint. If a factor is significantly associated with the endpoint, but does not influence the association of interest, then it does not, by definition, confound the association and should be excluded from the model. Including it would only make the model more complicated, less stable, and produce results that would be harder to interpret.

Many factors can influence the immune response. Furthermore, several environmental circumstances, such as hygiene and socioeconomic status, can considerably affect clinical endpoints. When one thinks that one of these factors could also be associated with the exposure to the xenobiotic under study, then one should consider it as a potential

confounder. If so, one has to evaluate such a factor, verify if it indeed confounds the association and, if needed, find ways to minimize the confounding effect.

Exposure assessment and classification

Exposure assessment

Measurement of exposure and classification of participants in exposure groups are central to human immunotoxicology. The challenges in evaluating the exposure are similar in human studies and in animal studies, although exposure assessment in humans must remain non-invasive. In human immunotoxicology, the key aspect of exposure assessment is a strong and detailed hypothesis because measurement and assigning of exposure will both be planned according to the stated hypothesis. The hypothesis should detail to what extent the dose and the length of exposure time are related to the toxic effect. Data collection and analyses are then performed accordingly. If the hypothesis states that it is the dose of exposure that is crucial in determining the toxic effect, and that the duration is less important, then the exposure assignment and the statistical analysis should be performed mainly on the dose of exposure. Of course, more than one type of analyses could be carried out. For example, one could analyze the results in terms of the duration of exposure, various expressions of dose (cumulative, peak, etc.) and all sorts of interaction between them. Specific and thorough knowledge of the xenobiotics under study is crucial in determining how to deal with exposure assessment.

Exposure classification

Again, the hypothesis is central in the decision of exposure assignment (who is considered exposed, and who is not). One willing to test the hypothesis which states that ‘cannabis use reduces the macrophage ability to secrete cytokines’ will have nothing to work with when the decision of who is considered exposed to cannabis will have to be made. When the hypothesis is clearly established, such as ‘smoking cannabis at least once a day for more than one year reduces cytokine secretion by lung macrophages’, the assignment of exposure is clear cut. In this example, everybody smoking cannabis less than once a day is unexposed. Similarly, somebody smoking cannabis more than once a day but for less than one year should also be considered unexposed. The definition of an induction period is important in determining to which group a subject belongs during the follow-up period. In

our example, the hypothesis states that there is a one-year induction time, i.e. that smoking less than a year should not be enough to induce an effect. This induction time should be considered when the contribution to the denominators of the exposed and unexposed groups is determined for each subject. Thus, the development of a clear hypothesis is essential. This is a scientific decision based on existing literature, not merely on statistics.

Human studies specificities

Factors influencing the immune response in humans

Several factors can influence the immune system response. Although many are comparable between humans and animals, some need to be dealt with distinctively in human studies. Only those factors will be addressed specifically in this section.

Among them is the large genetic variability of human populations as compared to laboratory animals. The variations of immune responses among human individuals will be proportional to the genetic heterogeneity in the population under study. Examples of this include the vaccination responses to measles and to hepatitis B, which are strongly influenced by the human leukocyte antigen (HLA) genotype, and the influence of the promoter region of several cytokine genes on the inflammatory response (see Bidwell et al. 1999, Howell et al. 2002, Kay 1996, and Van Loveren et al. 2001 for more information). It would be impractical and unnecessary to measure such genetic variations in all epidemiological studies. Although they greatly contribute to inter-individual variability, and thus to statistical power, they do not necessarily introduce biases. As with other factors affecting the immune system, the important aspect is to determine if they are potentially associated with the exposure under investigation in the study population. If so, they need to be considered as potential confounders (see section 2.2 on confounding). If not, the random error created by inter-individual variability can be minimized by recruiting an appropriately large number of subjects.

Important environmental factors influencing the immune response in humans include malnutrition, stress, age, smoking habits, exercise, diet, lifestyle and radiation. For a study to be valid, it is imperative that the investigator takes into account these factors in the study design, and in the analyses and interpretation of results.

Malnutrition is the commonest cause of immunodeficiency (Chandra 2002). Although severe malnutrition is not prevalent in developed country, the issue must often be addressed since only a small imbalance between energy intake and expenditure, as well as single-nutrient deficiencies could affect many pathways of the immune response (Chandra 2002, Marti et al. 2001). Malnutrition itself is delicate to investigate, but a good estimate of the socioeconomic status is critical and should always be considered.

Psychologic stress and stress hormones such as glucocorticoids and catecholamines can modulate the immune response. Stress can decrease immune endpoints by affecting white blood cell counts, immunoglobulin (Ig) levels, and antibody response to vaccination (see the following reviews by Cohen et al. 2001, Elenkov & Chrousos 2002, Herbert & Cohen 1993). Long-term and short-term exposures to stress can be tricky to evaluate and one will often rely on random distribution of subjects between the different groups under study. However, stress exposure can be of particular concern in occupational studies, especially when the exposure to a xenobiotic is strongly associated with the type of occupation, which in turn can be associated with occupational stress.

Age is a critical factor, as most immune functions will change with aging. The fetus and the neonate are unable to react to several foreign substances and have less efficient neutrophils compared to healthy adults (Wilson 1986). Aging adults have impaired T-cell and B-cell mediated responses, and a modification of their repertoire that could contribute to the development of autoimmunity (Antonaci et al. 1987, Pawelec & Solana 1997, Urban et al. 2002).

Several lifestyle-related factors have an influence on the immune system. Smoking affects leukocyte counts (Burton et al. 1983), recreational drugs could reduce cell immunity (Roth et al. 2002), and UV radiation affects hypersensitivity reactions and NK cell activity (Sleijffers et al. 2002). On the contrary, exercise slows down immune senescence (Pedersen et al. 2000, Venjatraman & Fernandes 1997), and vitamin A and fatty acids are important contributors of the immune response (Gil 2002, Grimm et al. 2002, Semba 1994). While most of the above mentioned factors can be measured or estimated, an evaluation of all lifestyle factors is financially and technically prohibitive. Depending on the type of outcome that is to be evaluated, one can rely on careful subject selection to minimize their

effects. A thorough identification of lifestyle factors potentially associated with the exposure to the xenobiotic under study will also help reducing the number of factors to evaluate. Importantly, the influence of lifestyle factors on any epidemiological outcome should not be underestimated. The alteration that they produce can often be greater than that of occupational and environmental exposure to xenobiotics. Unknown and unmeasured factors are unavoidable and could likely bias the results. This always needs to be taken into account when positive or negative results are interpreted.

Clinical immunodeficiency, preexisting diseases and medication use

The functional immune system will react to the incursion of a pathogen in the body and many dormant immune parameters will become activated in the presence of an infection. Extrinsic induction of immune and inflammatory processes by trauma, previous infection and vaccination are also frequent in human populations. On the other hand, some microorganisms have the ability to suppress the immune system. All these variations in immune function can greatly affect the study results. For example, the prevalence of the BCG vaccine could severely bias an endpoint such as the response to the tuberculin test (Van Loveren et al. 2001). A small difference in the prevalence of an immunosuppressing microorganism, such as the HIV, could also significantly bias the final results of a study. These are obvious examples, but one must keep in mind that many individuals have asymptomatic acute or chronic infections that can weigh in the immune balance in an unpredictable way.

Particular to human studies is the widespread use of immunomodulating drugs, predominantly the non-steroidal anti-inflammatory drugs (NSAIDs). Corticosteroids, NSAIDs, cyclophosphamide, and cyclosporine are all substances specifically designed to interfere with the normal immune response. The easiest way to eliminate the effects of pharmaceutical drugs is to ensure that the subjects under study abstain from any medication for a specified period prior to and during the course of the investigation, if possible. Medication use could be considered in statistical models but it is not always simple to deal with the doses and the interactions among several drugs. Furthermore, study participants will usually remember the prescription drugs they have taken, but could easily forget

sporadic ibuprofen use. This is to be considered, especially when acute inflammatory endpoints are measured.

Assays in human immunotoxicology

The immune system is functionally and structurally complex. It is an integrated arrangement of several tissues and organs working together. It has great functional reserves and numerous overlaps and back-ups. One must always keep in mind that when an 'abnormal' pathway is present, it may coexist with other 'normal' pathways. Therefore, normal findings do not exclude abnormal functions, which can be compensated by other pathways and thus remain clinically silent. It is accepted that on an individual level, the immune system of a healthy adult can support some level of insult without affecting host resistance. Nevertheless, on a population level, it is plausible to assume that even small impairments of the integrity of the immune function could have an effect on some vulnerable individuals. Consequently, the 'threshold' relationship observed on the individual level may not hold in a population, in which a more linear relationship between immunotoxicity and health status can be expected (Kimber & Dearman 2002, Luster et al. 1993). In this context, it has been argued that the so-called immune reserve should not be considered in the interpretation of population immunotoxicological data (Kimber & Dearman 2002, Selgrade 1999).

Unless one seeks to identify an effect on a very specific pathway, the immune system should always be regarded as a whole and its investigation should be done accordingly. We favor testing schemes in which most of the main immune subsystems are investigated simultaneously. Four such schemes have been elaborated by different groups (reviewed by Tryphonas 2001). They are summarized in table 2.1. The most complete scheme is the one proposed by the United States National Academy for Sciences (1992). It should be noted that to our knowledge, none of these schemes have been used as a whole. It is therefore difficult to predict their ability to detect immunotoxic effects in human populations.

Table 2.1
Proposed testing schemes for assessing immunotoxicology in humans

Assays	WHO*	CDC*	USNAS* [a]			Colosio et al.* [a]		
			1st tier	2nd tier	3rd tier	1st tier	2nd tier	3rd tier
A. Blood count and clinical chemistry								
Complete blood count with differential counts	✓	✓	✓			✓		
Clinical chemistry	✓	✓						
B. Inflammation and non-specific immunity								
C-reactive protein	✓	✓						
NK cells	✓			✓		✓		
NK cell function					✓			
Phagocytosis assay	✓							
C. Cellular immunity								
Primary delayed-type hypersensitivity reaction	✓			✓				
Secondary delayed-type hypersensitivity reaction	✓		✓					
Surface analysis for CD4, CD8, CD3 and CD20	✓	✓	✓			✓		
Other T- and B-cell markers (CD5, CD11, CD16, CD19, CD23, CD64, class II MHC)				✓				
Class I and II MHC antigen typing				✓				
Proliferative response to mitogens					✓			
Serum levels of cytokine				✓				
Cytokine production (*in-vitro*)								✓
D. Humoral immunity								
Immunoglobulin concentration in blood	✓	✓	✓			✓		
Antibody to ubiquitous antigen	✓		✓					
Primary response to protein antigen	✓		✓	✓				
Primary response to polysaccharide antigen			✓	✓				
Secondary response to protein antigen	✓							
Auto-antibody titers	✓	✓	✓			✓		
Proliferation to recall antigen	✓						✓	
IgE to allergen	✓							
Immunoglobulin subclass					✓			
Antiviral titers					✓			
Polyclonal immunoglobulin production *in-vitro*							✓	

* Testing schemes proposed by: WHO = World Health Organization (1996); CDC = United States Center for Disease Control and Agency for Toxic Substances and Disease Registry (World Health Organization 1996); USNAS = United States National Academy of Sciences (1992) and Colosio et al. (1999).

[a] The proposed schemes included a 3-tier approach in which the tests in the 2nd and 3rd tiers are done if abnormalities are detected in the 1st tier or 2nd tier, respectively. These tests could also be performed on subgroup of the population included in the 1st tier.

Total and differential blood counts

The determination of total blood count (TBC) with differential absolute counts constitutes a good, cheap, easily available starting point. Although TBC is a poor predictor of host resistance and has a low concordance with other immune response tests (Luster et al. 1993, Luster et al. 1992), it allows the identification of significant decreases in cells responsible for immune response. TBC tests are usually well standardized but are unfortunately easily influenced by several factors such as age, sex, infections and lifestyle. Nevertheless, since they will be critical in the interpretation of other functional tests, they should always be performed in all participants. They should not, however, be considered sensitive (Rose & Margolick 1992, United States National Academy of Science 1992, World Health Organization 1996).

Inflammation and non-specific immunity

Neutrophil and monocyte function

When fresh blood is available, the chemotaxis and respiratory burst of polymorphonuclear leukocytes can be evaluated. The most standardized method is the nitroblue tetrazolium dye reduction used to evaluate the respiratory burst (Fernandes & Queiroz 1999). More recent methods using flow-cytometric techniques can also be used for rapid evaluation of phagocytosis, respiratory burst, activation and bacterial killing of neutrophils and monocytes (Fruhwirth et al. 1998, Prodan et al. 1995, Salih et al. 2000). Warnings have been issued against the reproducibility of bacterial killing assays (World Health Organization 1996).

Complement

Alterations of the basal levels of complement proteins can be misleading and hard to interpret. A more useful approach is the measurement of the complement hemolytic activity of the classical (CH_{50}) and alternative pathways (AP_{50}) (Servais et al. 1991, White et al. 1986). The evaluation of complement components and function is important when complement deficiency is suspected clinically (for example a suspicion of increased incidence of systemic lupus erythematosus or of recurrent infections by *Neisseria* species and pyogenic organisms). Further analyses of complement function should be left to research teams specializing on the complement system.

Natural-killer cells

NK cells contribute to the immune response in the early phase of infection. They are very potent cells acting along non-specific pathways, independently from the major histocompatibility complex (MHC) (Janeway & Travers 1996, Trinchieri 1989). NK cells are lymphocytes expressing the CD3-CD16 and/or CD56 surface markers. The gold standard for the evaluation of their activity is the ^{51}Cr-release assay, in which K562 target cells are cultured with freshly isolated NK cells from peripheral blood (Laso et al. 1997, Trinchieri 1989). Other methods using fluorescence instead of radioactivity have been developed (Kantakamalakul et al. 2003, Piriou et al. 2000).

Cellular immunity

Immunophenotyping of lymphocytes

The development of flow-cytometric techniques has allowed the rapid phenotyping of lymphocytes for many surface antigens. Most published work on human immunotoxicology recommends a surface analysis of lymphocytes, at least for CD4, CD8, CD3 and CD20. Alterations of lymphocyte subpopulations, whether they are reductions of specific sub-populations or imbalances between two or more sub-populations, can yield valuable results. It was shown that the variation of surface markers had a good predictive value for other immune function tests (Luster et al. 1992) and for biologically relevant *in vivo* effects in mice (Luster et al. 1993). However, investigators should be aware that lymphocyte populations vary greatly with age. Historical controls exist (Babcock et al. 1987, Erkeller-Yuksel et al. 1992) but age must be considered in the design and the analysis when lymphocyte phenotyping is performed. It is noteworthy to mention that most data provided by flow cytometric analysis have a broad distribution, and it has been argued that such analyses are not warranted in human immunotoxicologic studies (Van Loveren et al. 1999). The same authors also mentioned the difficulty to predict the biological significance of the observed differences in lymphocyte subpopulations, which is often true. The phenotyping of lymphocytes can provide valuable information, but quantitative thresholds of T-cell subsets that are indicative of clear immune competence remain to be determined (Ward 1992).

Delayed type hypersensitivity reactions

The overall function of the intricate cell-mediated immunity (CMI) can be evaluated *in vivo* by delayed-type hypersensitivity (DTH) skin tests. The development of the CMI Multitest system (Merieux, France) has helped to standardize the antigen potency and the technique of administration (Rosenstreich 1993). The CMI Multitest allows the simultaneous intradermal injection of 7 common antigens and a glycerin control. The measurement of induration 48 hours after injection provides information on the ability of the CMI to respond to previously encountered antigens. This test has been used on several occasions and results for healthy subjects are available in the literature (Corriel et al. 1985, Hickie et al. 1995, Kniker et al. 1985, Moesgaard et al. 1987, Murgueytio & Evans 1988, Rosenstreich 1993).

The DTH response is sensitive to the usual factors affecting the immune response. Frequency of previous contacts with the antigens included in the Multitest must also be accounted for (prevalence of tuberculosis, immunization coverage, etc.). Geographic and ethnic variations exist and results should always be compared to a control group rather than to previously published values. The use of DTH response in children less than one year old is not warranted.

While the usefulness of well-performed DTH skin tests is not questioned, the standardization of administration and reading can be quite challenging. Well-trained staff can achieve high reproducibility between observers and retested participants (Frazer et al. 1985). Unfortunately, inter-reader variability can lead to unreliable results, as noted for an important proportion of subjects in a study on the effects of dioxin exposure (Hoffman et al. 1986). Furthermore, it has been argued that since the reaction elicited by the Multitest system are secondary reactions, the uncertainty of prior antigen exposure limits the usefulness of DTH skin reaction (Van Loveren et al. 1999). Unless well-trained and experimented staff is available, DTH testing may not be the best choice when subtle immune effects are investigated.

Lymphocyte proliferation

The proliferative response of peripheral blood leukocytes to mitogens or specific antigens is the *in vitro* correlate of DTH skin test. Substances such as phytohemagglutinin and

concanavalin A will induce lymphocyte proliferation, which can be measure by ^{3}H-thymidine incorporation assay (Rose et al. 1992), or by more recent techniques avoiding the use of radioisotopes (Maino et al. 1995, Schoel et al. 1996, Sottong et al. 2000).

Quantification of cytokine production by stimulated peripheral blood cells is useful for pinpointing mechanisms of action. It should not be used for the screening of immunotoxic effects, unless a precise mechanism is proposed.

Humoral immunity

Serum immunoglobulin concentrations

Determination of Ig levels is not a sensitive method because immunodeficiencies can be observed in the presence of normal Ig levels. In non-human primates, exposure to PCBs produced no effect on serum Ig while the ability to respond to a foreign antigen was impaired (Tryphonas et al. 1991a). Determination of serum immunoglobulin concentrations is not warranted unless very specific mechanisms are sought, or when increased incidence of Ig-mediated pathologies, such as hypergammaglobulinemia, are suspected (IUIS/WHO working group 1982, 1988).

Antibodies to ubiquitous antigen

Evaluation of antibodies against widely occurring antigens allows the identification of profound defects in antigen-specific Ig production in individuals with otherwise normal Ig levels. An example of this is the Wiskott-Aldrich syndrome in which subjects lack isohemagglutinins but have normal Ig levels. Absence of natural antibodies to blood group antigens and to *Escherichia coli* can easily be identified by simple agglutination tests. This type of investigation should be performed only when an immunotoxic mechanism severely affecting the humoral response is suspected. It is not sensitive and will most likely yield deceiving results when subtle effects are sought.

Antibodies to self-antigen

The identification of clinically relevant autoimmunity is complex. Autoimmune diseases are diagnosed based on biologic and clinical criteria and the significance of the presence of autoantibodies in asymptomatic individuals is not clear (Holsapple 2002, Vial et al. 1996). Other tests exist in animal models but they cannot be used in human studies (Pieters et al.

2002, Vos & van Loveren 1995). Because autoantibodies are present in healthy individuals, and because the significance of their presence is poorly understood, it is not warranted to use them as biomarkers of autoimmunity without other clinical markers (Descotes et al. 1995, Holsapple 2002).

Antibody response to immunization

The evaluation of antibody development following vaccination is the gold standard of human immunotoxicology. The immune system needs proper antigen processing and presentation, as well as functional B-cells and T-cells in order to mount an appropriate response to a protein antigen. In rodents, assessment of an antibody response was shown to be the most adequate indicator of immunotoxicity (Luster et al. 1993, Luster et al. 1992). The determination of response to immunization also offers the opportunity to improve public health by increasing the vaccination coverage in the population under study.

Generally, investigators seem to agree to the fact that the primary response is more sensitive than the secondary response for assessing insults to the immune system (Rose & Margolick 1992, Van Loveren et al. 1999, World Health Organization 1996). However, a review by Cohen et al. (2001) on the effect of stress on response to immunization underlined a more stable effect reported by investigators using the secondary response as the endpoint. Different pathways are at play in primary and secondary responses and both should be tested whenever possible.

When antibody response to immunization is investigated, it is essential to evaluate serum antigen-specific antibody before and after the challenge. Unknown previous encounters or challenges with the antigen for some participants can greatly influence the results. Sequential assessments would also be useful to determine the catabolic rate of specific antibody, as well as Ig isotypic class switching. Vaccination response is influenced by the type of vaccine, the route of administration, and the time elapsed between the challenge and the assessment of the response (Van Loveren et al. 2001). Other factors such as age, genetics, stress, smoking, nutrition, and some infections also affect the response to vaccination. All these factors should be considered as potential confounders when vaccination response is used as an endpoint (Van Loveren et al. 2001).

When possible, vaccines offering public health advantage should be preferred. Synchronization with childhood vaccination programs can be particularly effective when immunotoxic effects in children are investigated. In adults, hepatitis B vaccine could be used, but the increasing immunization coverage begins to limit its utilization as a marker of effect for primary response. The use of harmless, but immunogenic antigens foreign to humans, such as the bacteriophage phiX174 (Rubinstein et al. 2000) and the keyhole limpet hemocyanin (Harris & Markl 1999), can be of great value. These two antigens can also be used to evaluate the anamnestic response by re-challenging participants. Polysaccharide vaccines, for example the pneumococcal vaccine, are useful for the evaluation of T-cell independent response. Despite the usefulness of this marker of effect, very few investigators have used it in human immunotoxicology studies (reviewed by Van Loveren et al. 2001).

Clinical endpoints

All of the methods mentioned above will allow the identification of alterations in one or more pathways of the immune system. When such alterations are proven or suspected, one needs to evaluate if these alterations affect the health status of a population. Since the ultimate role of the immune system is to maintain the integrity of the body by dealing adequately with the multitude of threats coming from inside and outside, any alteration of the immune system could potentially result in loss of this integrity. This can happen because the immune system does not fight hard enough, fights too much, or begins to forget whom to fight, and whom not to fight.

In addition to the factors affecting the immune system discussed above, other risk factors for the clinical endpoint under study, not necessarily related to the immune system, will need to be considered as potential confounders. For example, the incidence of infectious diseases is affected by the frequency of contacts with pathogens (daycare attendance, crowding), education, vaccination coverage, breastfeeding, and access to medical services. Exposure to xenobiotics is often related to the socioeconomic status, which in turn can affect many of the above-mentioned factors. A thorough knowledge of all the factors affecting directly, or indirectly the studied endpoint is critical.

In this section, we will discuss some clinical endpoints in relation to immunotoxicology, with a focus on infection susceptibility. We did not attempt to cover all methods and all potential clinical endpoints of interest in immunotoxicology. This section should be regarded as a starting point when one considers the inclusion of such endpoints in a study. The participation of expert epidemiologists, researchers and clinicians in the area of interest remains essential.

Infections

Contrary to cancer and other chronic diseases, acute infections have a very short latency period. As soon as the immune system is compromised, the risk of having acute infectious episodes increases. This aspect is not to be overlooked. Much effort is dedicated to the design of early biomarkers of effect, but infection incidence should actually be considered as such. Because the evaluation of infectious disease frequency is relatively easy to perform, such an endpoint should be included whenever possible.

Many factors affect the infection incidence. In particular, the socioeconomic status of the individuals but also of the population as a whole, strongly influence the risk of having infections. Factors such as education, hygiene, crowding, nutrition, and access to health services are often uneven in populations, and are sometimes strongly correlated with exposure to environmental and occupational xenobiotics. Example of this is the important exposure to dioxin experienced by the inhabitants of the Quail Run Mobile Home Park in Missouri (United-States) following the spraying of dioxin-contaminated oil near their homes (Hoffman et al. 1986). The low socioeconomic status of the exposed participants represented a challenge for the investigation of immunotoxic effects. The recruitment of a control group of participants living in similar homes and conditions enhanced the credibility and validity of the study results.

Even in populations for which there is no suspicion of immune deficiencies, there is still a background of acute and chronic infections of all sorts. If immune alterations are suspected in a population, one could expect an increase in the incidence of infections, an increase in the severity of these infections, or both. The central question in measuring infections in human immunotoxicology is the identification of a difference in incidence or severity

between an exposed group and an unexposed group. Often, incidence and severity are entangled with each other as an increase in the severity of an otherwise benign infection will facilitate its identification in a population and, consequently, increase its observed incidence. In this section, we will focus our discussion on increased incidence.

It is sound to expect that a group with a higher incidence of a given infection will also have a higher prevalence and a higher risk of developing the disease in a given time frame. The evaluation of prevalence (*x* persons having the disease out of *y* participants at a given time) and average risk (*x* persons having one or more episodes out of *y* participants during a given period of time – also called incidence proportion) are useful shortcuts. However, the most straightforward method is to try, when it is possible, to directly evaluate the incidence.

Incidence of acute infections

Ideally, to evaluate the incidence of, let us say middle-ear infections, the investigator should follow a group of healthy participants regularly in the hope of diagnosing middle-ear infections as these become identifiable. Of course, it is seldom possible in large cohorts to visit the participants, or to ask them to visit the clinic every week. Less frequent follow-ups during which the participants are asked if they have been diagnosed for middle-ear infections since the last follow-up is a good alternative. By doing so, the investigator has to deal with episodes that are recalled and reported by the participants instead of episodes diagnosed by a physician, therefore opening the door to recall biases. The date of onset of the episode is also less precise.

For benign infections, a questionnaire or a self-maintained log in which participants record daily or weekly their symptoms related to the infection under study could be quite useful. When the definition of the symptoms is clear and complete, this method could yield valuable results with accurate dates. This method is efficient with common colds and upper respiratory tract symptoms, provided that only self-reported symptoms will be available.

A thorough review of the medical charts can be used to evaluate incidence. The medical chart review is usually easier to perform in remote rural areas where most of the population members attend the same health center. The principal advantage of the medical chart review is that dates of diagnoses are available and several pathologies can be evaluated at

the same time. However, only infections for which medical attention was sought will be detected, and diagnoses are usually not standardized. Benign infections could be missed when participants decide not to go to the clinic, a decision that is often related to other risk factors such as socio-economic status, therefore potentially introducing a bias. Asking the participants to go to a specific clinic when they have symptoms, and having the physicians of that clinic participate in the study will help to standardize the disease definitions and reduce the number of participants who decide not to consult for their symptoms.

The approach of the medical charts review was recently used by our group to evaluate the effect of OCs on infection incidence (Dallaire et al. 2004a). We reviewed the medical charts of 199 Inuit infants during the first months of life and used Poisson regression to evaluate the association between the incidence of acute infections and prenatal exposure to OCs. We found that the infants in the higher exposure groups (2^{nd}, 3^{rd}, and 4^{th} quartiles) had an increased incidence of acute infections such as otitis media, compared to the infants in the lower exposure group (1^{st} quartile).

The combination of more than one of the methods mentioned above is warranted. Crosschecking self-reported episodes with medical charts helps in rectifying dates and standardizing diagnoses.

Prevalence and average risk of acute infections

When it is impossible or impractical to evaluate the incidence of infection, other measures of disease frequency, such as prevalence and average risk (incidence proportion), can be used. The biggest advantage of prevalence is the need for only one assessment for each participant, therefore eliminating the infrastructure necessary to follow-up participants. In this context, it is easier to thoroughly examine each participant in order to make clear standardized diagnoses. This approach was used by Chao *et al.* (1997) for the evaluation of children pre-natally exposed to PCBs in Taiwan. In this study, two otolaryngologists examined each child for ear abnormalities and a diagnosis was made when the two agreed. Unfortunatly, prevalence cannot be used when the duration of the disease is short. The cross-sectional nature of the data also renders them subject to the warnings discussed in section 3.1 on study designs.

Average risk is usually the measure obtained when questionnaires are used. Questions such as '*has a doctor ever given your child a diagnosis of otitis media*' (Weisglas-Kuperus et al. 2000) will yield average risk (*x* % of the participant who ever had otitis media since they were born). Average risk is also the measure obtained by Dewailly *et al.* (2000) in a study assessing the effect of OCs exposure on middle-ear infections. To evaluate the number of episodes in the first year of life, the authors conducted interviews and medical examinations at 3, 7, and 12 months of age, during which they asked the mothers for the occurrence of any previous episodes. Then, they cross-checked the self-reported episodes in the medical charts of the infants and computed the risk of having ≥ 1 episodes, and ≥ 3 episodes. Because this type of evaluation has a dichotomous outcome, the investigator has to determine, sometimes arbitrarily, a threshold value. This has the disadvantage of potentially reducing the statistical power of the study as compared to continuous outcomes.

Chronic and opportunistic infections

The methods discussed above can be applied to chronic or opportunistic infections. Case-control designs represent also an interesting alternative when the disease studied is less frequent. There exist many other methods for identifying participants with the disease under study. The use of available standardized databases can be of great value in some settings.

Other clinical endpoints

Allergy

Clinically defined allergy can usually be assessed using the same strategy as infections. As with infection, prevalence and average risk will be easier to obtain than incidence. Whenever a questionnaire is administered, simple questions about allergies such as '*has your child ever had eczema or an allergic reaction*' should be asked (Weisglas-Kuperus et al. 2000). So far, the search for efficient biomarkers of allergy that could be applied to large cohorts has been deceiving (Odelram et al. 1995).

Autoimmunity and neoplasic changes

Autoimmunity can be briefly defined as the loss of immune tolerance for auto-antigens. The sole presence of autoantibodies does not mean that symptoms of autoimmunity will be

present. Since no clear biomarkers of autoimmunity are known, and because the diagnosis of clinical autoimmunity is complex, studies focusing on clinical autoimmunity should be left to teams specialized in the subject.

Although it has long been observed that immunosuppression was linked to increased frequency of cancer (Spector et al. 1978), the hypothesis of a reduced surveillance of tumors by immonudeficient individuals is controversial (Newcombe 1992). It is unlikely that cancer will be the first manifestation of immunotoxicity. Screening for increased cancer incidence is not warranted for the purpose of identifying early immunotoxic properties of an exposure. However, a large population exposed to a known immunotoxic agent could be at increased risk, and investigation of links between development of cancer and exposure might be an interesting research avenue. On the other hand, observation of an unexplained increase of cancer rate in a population should elicit researchers to consider an immunotoxic etiology.

It is noteworthy that researchers in France have initiated a sentinel program in which autoimmune diseases and non-Hodgkin lymphomas are screened in order to flag higher than expected incidences associated with chemical exposure (Descotes et al. 1996). The hypothesis that can spur from this approach could be interesting to pursue.

Research needs

Useful standardized non-invasive approaches exist for the assessment of immunotoxicity in human populations. However, relatively few studies have been conducted in human settings and the methods varied greatly. Unfortunately, the proposed testing schemes have hardly ever been used and their ability to detect immunotoxic effects has yet to be established. The field of human immunotoxicology would greatly benefit from the widespread use of recognized schemes of assays by several researchers on several suspected immunotoxic agents. Furthermore, the relation between biomarkers and clinical endpoints is still obscure in humans. The design of studies in which both clinical and biologic endpoints are assessed is greatly encouraged. Only these studies, if they are well designed and conducted, will allow the identification of relevant markers with high specificity and sensitivity to predict adverse effects on the immune system in human populations. Finally, the assessment of

acute clinical events, such as the incidence of infections, remains a relevant and easily evaluated endpoint that should be used as often as possible in human population studies.

Acknowledgements

We thank Daria Pereg for her critical review and useful inputs during the preparation of the manuscript.

Chapitre 3 – Tendances temporelles de l'exposition prénatale aux OC et aux métaux lourds chez des enfants inuits du Nunavik entre 1994 et 2001

Dallaire F., Dewailly E., Muckle G. et Ayotte P. 2003. Time trends of persistent organic pollutants and heavy metals in umbilical cord blood of Inuit infants born in Nunavik (Québec, Canada) between 1994 and 2001. *Environ Health Perspect* 111(13):1660-1664.

Résumé

Les habitants du Nunavik (Québec, Canada) consomment de grandes quantités de produits marins et sont conséquemment exposés à des doses relativement élevées de contaminants environnementaux s'accumulant dans la chaîne alimentaire. Dans cette étude, nous étudions les tendances temporelles des polluants organiques persistants, du mercure et du plomb dans le sang de cordon ombilical d'enfants vivant dans trois communautés de la côte est de la Baie d'Hudson au Nunavik. Nous avons analysé 251 échantillons de sang de cordon ombilical recueillis de 1994 à 2001. Nous y avons dosés les biphényles polychlorés (BPC), le dichlorodiphényle trichloroéthane (DDT), le dichlorodiphényle dichloroéthylène (DDE), l'hexachlorobenzène (HCB), les chlordanes, le plomb et le mercure. En utilisant un modèle exponentiel, nous avons identifié une tendance descendante fortement significative pour les BPC (7,9 % par années , $p < 0.001$), le DDE (9,1 % par année, $p < 0.001$), le DDT (8,2 % par année, $p < 0.001$), et le HCB (6,6 % par année, $p < 0.01$). Nous n'avons pas détecté de tendance significative pour les chlordanes. Une réduction significative du plomb et du mercure ont été observée, mais il n'y avait pas de relation linéaire ou exponentielle claire. Les diminutions de concentrations d'OC observées pourraient être expliquées par une diminution de la contamination de la nourriture, par des changements dans les habitudes alimentaires, ou, plus probablement, par une combinaison des deux phénomènes.

Abstract

Inuit inhabitants of Nunavik (northern Québec, Canada) consume great quantities of marine food and are therefore exposed to high doses of food chain contaminants. In this study, we

report the time trends of persistent organic pollutants, mercury, and lead in umbilical cord blood of infants from three communities of the east coast of Hudson Bay in Nunavik. We analyzed 251 cord blood samples collected from 1994 through 2001 for polychlorinated biphenyls (PCBs), dichlorodiphenyl trichloroethane (DDT), dichlorodiphenyl dichloroethylene (DDE), hexachlorobenzene (HCB), chlordanes, lead, and mercury. Using an exponential model, we found strongly significant decreasing trends for PCBs (7.9 % per year, $p < 0.001$), DDE (9.1 % per year, $p < 0.001$), DDT (8.2 % per year, $p < 0.001$), and HCB (6.6 % per year, $p < 0.01$). No significant trends were detected for chlordanes. A significant reduction of lead and mercury concentrations was found, but there was no clear linear or exponential trend. The decreases observed could be explained by a decrease in food contamination, by changes in dietary habits, or, most likely, by a combination of both.

Introduction

Lipophilic organochlorines (OCs) that resist biodegradation can accumulate in the environment to become persistent organic pollutants (POPs). Among them, polychlorinated biphenyls (PCBs) and several chlorinated pesticides have been detected in tissues of animals and human throughout the world. Their capacity to accumulate in adipose tissue leads to biomagnification in the food chain, and their concentrations reach highest levels in top predator species (Braune et al. 1999, Muir et al. 1999). Mercury and lead are ubiquitous in the environment. They both occur naturally, but human activity has increased their mobilization and distribution in the environment. Mercury is excreted slowly by animals and plants and also accumulates in the food chain (found mostly as methylmercury, its organic form).

Most studies focusing on temporal trends of POPs have identified a decreasing trend during the last decades. Since the mid-1970, levels of dichlorodiphenyl trichlorethane (DDT) and PCBs have decreased in tissues of freshwater fishes and Herring Gull eggs in Canada and the United States (Hebert et al. 1994, Ryckman et al. 1994, Schmitt et al. 1999). In Arctic wildlife, POP concentrations seem on the decline for most species (Muir et al. 1999, Muir & Norstrom 2000), but not all (Muir et al. 2000). In environmentally exposed humans, levels of PCBs in breast milk have dropped in the last 15 years in Germany (Schade & Heinzow 1998) and Sweden (Noren 1993, Noren & Meironyte 2000). Downward trends in

human fluids have also been observed in Michigan (He et al. 2001), Mexico (Waliszewski et al. 1998), Canada (Dallaire et al. 2002), and the United Kingdom (Harris et al. 1999).

For cultural and economic reasons, carnivorous fish and marine mammals constitute an important part of the diet of the inuit population living in Nunavik (northern Quebec, Canada). Their exposure to such biomagnified substances as OCs and heavy metals is thus proportionally high. Several studies have identified markedly higher mean concentrations of POPs and heavy metals in adult blood, cord blood, and breast milk of Nunavik inhabitants compared with those of the southern population of the province of Québec. (Ayotte et al. 1997, Dewailly et al. 1993, Dewailly et al. 1998a, Dewailly et al. 1989, Muckle et al. 1998, Rhainds et al. 1999). In this context, we report here the temporal variations of POPs, mercury and lead in umbilical cord blood of infants born from 1994 through 2001 in three communities of the coast of the Hudson Bay in Nunavik.

Materials and Methods

Population and recruitment

The targeted participants were pregnant inuit women living in three communities (Puvirnituq, Inukjuaq, and Kuujjuaraapik) on the east coast of Hudson Bay in Nunavik (figure 3.1). For the present analysis, we included the participants of two previous studies done on the same population. The first study was designed to monitor the prenatal exposure to environmental contaminants and took place between November 1993 and December 1996 (Dewailly et al. 1998a). Pregnant inuit women were invited to participate on arrival at the health center for delivery. Of the 491 women who accepted to participate, only the women living in the three targeted communities (Puvirnituq, Inukjuaq, or Kuujjuaraapik) were included in the present analysis (n = 138). We excluded women living in other communities because we had a significant number of samples for only three years for these communities, and we believed that the interference caused by annual variations would have been too great for the estimation of valid time trends.

Figure 3.1. Geographic location of Nunavik, Québec, Canada

The second study was designed to evaluate the impact of environmental exposure to POPs and heavy metals on infant health and development (Muckle et al. 2001b). It was conducted between November 1995 and March 2002 in the three communities mentioned above. In this study, pregnant women were approached by one of our research assistants after their first prenatal medical visit. Three hundred and fifty-eight eligible pregnant women were approached, and 248 (69.3 %) accepted to participate. Because of staff shortage during deliveries, only 113 cord blood samples were available when statistical analyses were done. The participants for whom the cord blood samples were unavailable were similar to the other participants regarding gestational age, parity, maternal age, and infant sex. However, they had slightly lower maternal blood mean concentration of OCs. The 113 participants for whom cord blood was available and the 138 participants of the first study composed our study population for the present analysis (n = 251).

All women who agreed to participate to either study signed an informed consent form and could withdraw at any time. Both protocols were approved by the Laval University ethics committee.

Data collection

The data collection and all the laboratory procedures were rigorously the same for both studies. We sampled blood (10-20 mL) from the umbilical cord at delivery immediately after it was severed. An aliquot of blood was centrifuged, and the plasma was transferred into a glass vial prewashed with hexane. Blood and plasma were kept frozen at –80 °C and sent to the Institut National de Santé Publique du Québec (Québec City, Canada) every 3 or 4 months for contaminant and biochemical analyses. We gathered information on parity, maternal age, infant sex, gestational age, and birth weight from the medical charts of the mother and the newborn a few weeks after delivery.

Determination of OCs

Details on the procedures used by our laboratory, as well as information on the quality control of our chemical analyses, have been described previously (Rhainds et al. 1999). We determined the concentrations of 14 PCBs congeners (IUPAC numbers 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183, and 187) and of 11 chlorinated pesticides [aldrine, α-chlordane, γ-chlordane, *cis*-nonachlor, *p,p′*-DDE, *p,p′*-DDT, hexachlorobenzene (HCB), mirex, oxychlordane, *trans*-nonachlor and β-hexachlorocyclohexane] in plasma samples by high-resolution gas chromatography. Plasma samples (2 mL) were extracted, cleaned on Florisil columns, taken to a final volume of 100 μL, and analyzed on an HP-5890 series II gas chromatograph equipped with dual-capillary columns and dual ^{63}Ni electron-capture detectors (Hewlett-Packard, Palo Alto, CA, USA). We identified peaks by their relative retention times obtained on the two columns using a computer program developed in-house. The limit of detection was 0.02 μg/L for all OCs analyzed. Because OCs are stored mainly in body fat, the concentrations are expressed on a lipid basis.

Determination of blood lipids

We measured total cholesterol, free cholesterol, and triglycerides in plasma samples by standard enzymatic procedures. Phospholipid concentrations were determined according to

the enzymatic method of Takayama et al. (1977) using a commercial kit (Wako Pure Chemical Industries, Richmond, VA, USA). We estimated the concentrations of total plasma lipids using the formula developed by Phillips et al. (1989).

Determination of heavy metals

We determined total mercury concentrations by cold-vapor atomic absorption spectrometry. Samples were digested with nitric acid, and mercury was reduced by adding anhydrous stannous chloride ($SnCl_2$) and cadmium chloride ($CdCl_2$). Metallic mercury was volatilized and detected by atomic absorption spectrometry (model 120, Pharmacia, Piscataway, NJ, USA). The detection limit for blood mercury analysis was 1.0 nmol/L. We determined lead concentration by diluting an aliquot of whole blood with a mixture of nitric acid, ammonium phosphate, and Triton X-100. We then analyzed it by graphite furnace atomic absorption with Zeeman background correction (model ZL 4100, Perkin Elmer, Norwalk, CT, USA). The detection limit of the method was 50 nmol/L.

Determination of fatty acids in plasma phospholipids

For the determination of the fatty acid composition in plasma phospholipids, 200 µL aliquots of plasma were extracted after the addition of chloroform : methanol (2:1, vol/vol) in the presence of a known amount of internal standard (diheptadecanoyl phospholipid). We isolated total phospholipids from the lipid extract by thin-layer chromatography using heptane:isopropyl ether:acetic acid (60:40:3, vol/vol/vol) as the developing solvent. Following transmethylation, using BF3/methanol, the fatty acid profile was determined by capillary gas-liquid chromatography. The fatty acid composition of plasma phospholipids was expressed as percentages of the total area of all fatty acid peaks from C14:0 to C24:1 (Holub et al. 1987).

Statistical analysis

We assigned a value of half the detection limit of the analytic method when a compound was not detected in a sample. In all statistical analysis, we considered only those substances for which 50 % of the samples were above the limit of detection. This was done because trends calculated from a majority of samples with imprecise values (below the limit of detection) would most likely yield biased results. Contaminant concentration variables had

log-normal distributions and were log-transformed for all analyses. Therefore, all contaminants results are presented as geometric means. All the other continuous and discrete variables are presented as arithmetic means ± standard deviation. For temporal trends, we performed multiple regression modeling using the year of birth of the baby as the main independent variable. Because the dependent variables (contaminant concentrations) of the regressions were logarithmically transformed, the estimated slope of the regression (β) can be interpreted as the number of log increase (or decrease) of contaminant concentrations per year. From these results, we calculated the percentage of increase (or decrease) of contaminant concentrations per year using the expression: $[(1 - e^{\beta}) \times 100]$. To identify potential confounders, we selected variables that were associated with one or more contaminants and that were not constant in time. We did not consider dietary variables because our hypothesis included dietary changes as a potential explanation for temporal variations. We controlled for the village of residence, maternal age (continuous), parity (continuous), and the season of birth. We excluded birth weight and gestational age because these variables did not affect the association between contaminants and the year of birth. We also estimated adjusted means (least-square means) for each year using multiple regression in order to produce figures 3.2 and 3.3. In this case, contaminant concentrations in cord blood of infants born in 2000 and 2001 were merged because numbers were small in 2001 ($n = 5$). The database management and all the statistical analyses were performed with SAS software (SAS institute, Cary, NC, USA). By convention, we considered a $p < 0.05$ statistically significant.

Results

After merging of the data from the two studies, 251 participants could be included in the analysis. Concentrations of OCs, mercury, and lead were available for 238, 240 and 242 participants, respectively. Table 3.1 shows the descriptive characteristics of the participants. The mean age at delivery was 23.5 ± 4.5 years. About half of the participants were from Puvirnituq, 36.3 % were from Inukjuaq, and the remaining 11.1 % were from Kuujjuarapik (figure 3.1). Consistent with previously published data, maternal age and parity were strongly associated with most OCs in cord blood (data not shown).

Table 3.1
Descriptive characteristics of the participants

Characteristics	Arithmetic mean	Proportion
Mothers		
Age in years (*n* = 247)	23.5 ± 4.5	
Number of previous pregnancies (*n* = 248)	2.1 ± 1.8	
Residence (*n* = 251)		
Puvirnituq		52.6 %
Inulkjuaq		36.3 %
Kuujjuarapik		11.1 %
Newborns		
Sex (% of male) (*n* = 249)		52.2 %
Gestational age in weeks (*n* = 213)	39.2 ± 1.4	
Birth weight in grams (*n* = 245)	3513 ± 438	

Table 3.2 presents the unadjusted and adjusted annual decreases for contaminant concentrations when an exponential model is used. Except for heavy metals, the adjusted model yielded slightly steeper slopes and smaller *p*-values than did the unadjusted model. The concentration of the sum of PCB congeners decreased by 6.1 % per year in the unadjusted model ($p < 0.05$) and by 8.5 % per year in the adjusted model ($p < 0.001$). For individual PCB congeners, all trends were statistically significant, except for PCB 153 and PCB 170 in the unadjusted model.

Table 3.2
Annual percentage of decrease [a] of contaminants concentration in umbilical cord blood

Contaminants	% detected [b]	Annual decrease in percentage (95 % CI)	
		Unadjusted (n = 238) [d]	Adjusted [c] (n = 234) [e]
PCBs and pesticides			
ΣPCB congeners	-	6.1 (1.0 : 10.9)*	8.5 (3.7 : 13.1)#
PCB 99	94.6	7.9 (3.1 : 12.5)**	9.5 (4.7 : 14.1)#
PCB 118	90.5	7.1 (2.3 : 11.7)**	9.1 (4.4 : 13.6)#
PCB 138	100.0	5.7 (0.5 : 10.7)*	8.2 (3.2 : 13.0)**
PCB 153	100.0	5.1 (-0.4 : 10.4)	7.6 (2.2 : 12.7)**
PCB 170	80.2	4.5 (-1.6 : 10.2)	7.5 (1.9 : 12.8)**
PCB 180	99.2	8.1 (2.5 : 13.3)**	11.0 (5.9 : 15.8)##
PCB 187	95.4	5.4 (0.6 : 9.9)*	8.0 (3.7 : 12.1)#
DDE	100.0	6.7 (6.4 : 11.5)*	9.3 (4.4 : 14.0)#
DDT	71.5	5.9 (1.1 : 10.4)*	8.1 (3.6 : 12.5)#
HCB	100.0	4.8 (0.3 : 9.0)*	6.4 (2.1 : 10.6)**
cis-nonachlor	74.0	0.4 (-4.9 : 5.4)	0.9 (-4.4 : 5.8)
Oxychlordane	94.6	0.9 (-5.4 : 6.7)	2.8 (-3.5 : 8.6)
trans-nonachlor	100.0	2.3 (-2.8 : 7.1)	4.0 (-1.0 : 8.8)
Heavy metals			
Pb	100.0	8.2 (3.5 : 12.5)#	8.7 (4.5 : 12.7)##
Hg	100.0	9.4 (5.0 : 13.6)##	8.3 (3.8 : 12.7)#

[a] Percentage decrease per year calculated by multiple regression in which the year of birth is a continuous variable. Because the dependant variable (contaminants concentrations) of the regression was logarithmically transformed, each annual decrease and 95 % CI were calculated from the slope (β) of the regression estimate (and its 95 % CI) according to $[(1 - e^{\beta}) \times 100]$.
[b] Percentage of samples for which the contaminant was above the limit of detection.
[c] Adjusted for the village of residence, maternal age, parity, and season of birth.
[d] $n = 242$ for lead and $n = 240$ for mercury.
[e] $n = 237$ for lead and $n = 235$ for mercury.
* $p < 0.05$; ** $p < 0.01$; # $p < 0.001$; ## $p < 0.0001$.

For chlorinated pesticides, we detected significant declining trends in the concentrations of *p,p′*-DDE and *p,p′*-DDT. The decrease for *p,p′*-DDE concentration was 9.3 % per year in the adjusted model ($p < 0.001$). The ratio of DDT to DDE concentrations was stable across the entire study period (data not shown). HCB also decreased significantly by 4.8 % (unadjusted) and 6.4 % (adjusted). We could not detect any significant trends in chlordane concentrations.

Both mercury and lead concentrations decreased by more than 8 % per year for the study period. The trends were strongly significant, both for the unadjusted and for the adjusted models.

Figure 3.2 and 3.3 illustrate the temporal trends for OCs (figure 3.2) and heavy metals (figure 3.3). The solid lines represent the adjusted trends as presented in table 3.2. It is interesting to note that, although the exponential model for lead was significant, visual inspection of the data did not support an exponential decrease (figure 3.3). The mean concentrations were fairly constant until 1998 and sharply dropped in 1999. In fact, the fit of a statistical model contrasting the mean levels before and after 1999 (represented by the dotted lines) was better than the fit of the exponential model (solid line). Figure 3.3 also shows that mercury levels seemed constant in time, except in 1998 and 2000, despite what was found using an exponential model.

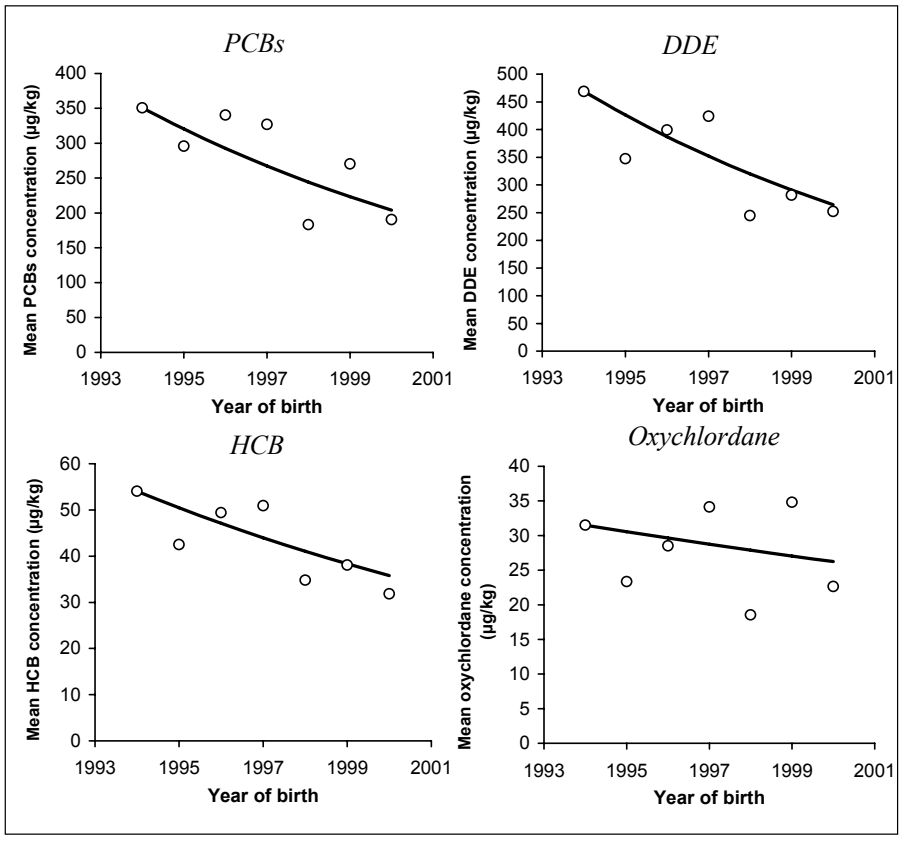

Figure 3.2. Adjusted mean OCs concentrations according to the year of birth. The solid lines represent the slope estimates presented in table 3.2.

We examined n-3 fatty-acid proportions as a surrogate of maternal fish consumption (Silverman et al. 1990) to see if a diminution of fish consumption had occurred during the study period. A slight decrease in n-3 fatty acids was detected, but it was not statistically significant. Expressed as a percentage of the total fatty acids, mean n-3 fatty-acid proportion was 4.8 % in 1994, and the model yielded a decrease of 0.06 % per year ($p < 0.28$).

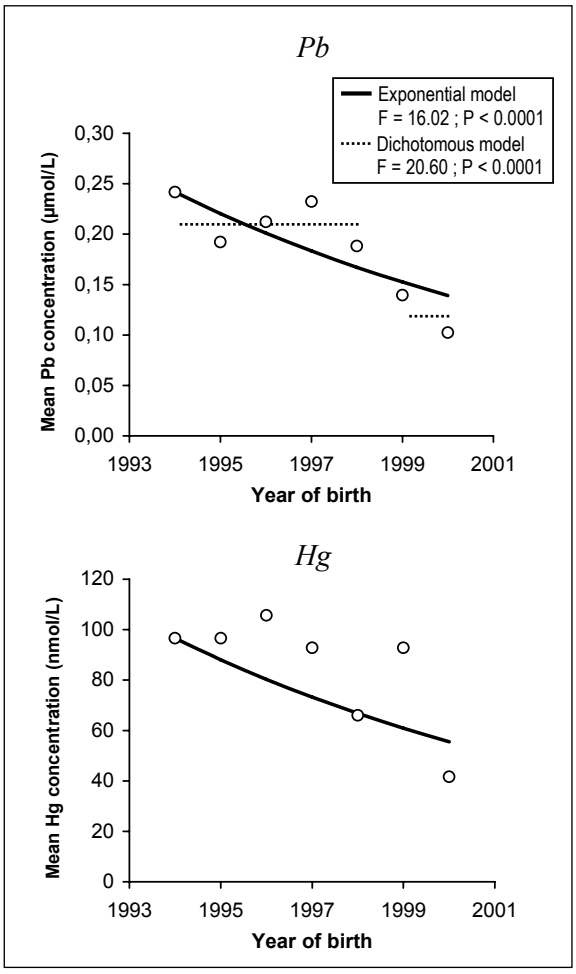

Figure 3.3. Adjusted mean heavy metal concentrations according to the year of birth. The solid lines represent the slope estimate presented in table 3.2. The dotted lines represent the adjusted geographic mean lead concentrations before and after January 1999.

Discussion

In this study, we identified an exponential decrease in most of the contaminants analyzed. Knowing that the major source of exposure in our study population is the ingestion of traditional food items of high trophic level, the decrease observed could be explained by a decrease in food contamination, by changes in dietary habits, or by a combination of both.

In Canada, the use of chlorinated pesticides and PCBs has been severely restricted since the 1970's. The use of most chlorinated pesticides is banned, and only closed-use PCBs in already existing equipment are still allowed. Today, it is believed that local sources of PCBs, mainly from abandoned contaminated arctic military sites, do not contribute significantly to human exposures in the Arctic. The comparison of PCB congener signature in soil showed that the impact of arctic contaminated sites was limited to their immediate vicinity, a halo of few kilometers (Bright et al. 1995). The situation is similar for chlorinated pesticides. They have been used in the 1940's and the 1950's for insect control in Nunavik but are unlikely to be used today because of regulations (although information on the use of pesticides in the arctic is scarce).

Nevertheless, release in the environment still occurs because of storage leakage and ongoing use in certain parts of the world. OC contaminants are distributed throughout the globe and reach arctic regions by long-range atmospheric transport and oceanic currents (Barrie et al. 1992, Burkow & Kallenborn 2000, Thomas et al. 1992). They accumulate in the food chain, and it is now well accepted that the high trophic level of the traditional inuit diet is mainly responsible for the high exposure of inuit populations to biomagnified substances such as POPs (Bjerregaard et al. 2001, Dewailly et al. 1993, Kuhnlein et al. 1995).

The eating habits of inuit populations have changed enormously during the last 50 years. Since market-bought food has been introduced in their diet, added carbohydrates, junk food, pork, chicken, milk products, and other "foreign" food items have become increasingly popular, especially among adolescents and young adults (Blanchet et al. 2000, Moffatt et al. 1994, Murphy et al. 1995, Olsen 1985). Market-food usually has a lower trophic level than does traditional inuit food and is consequently less contaminated by

POPs. This is reflected by the much lower mean concentrations of PCBs and chlorinated pesticides detected in cord blood samples from populations whose diet is almost exclusively composed of market-bought food, such as those in southern Quebec (Rhainds et al. 1999). However, although a gradual switch from traditional food to market food would result in a decrease in blood concentrations of food chain contaminants, it seems unlikely that dietary modifications would be of such magnitude that they alone would cause an annual decrease of 5-10 % in the body burden of contaminants.

Information on OC time trends in wildlife is scarce. Since the 1980's, there seems to be a decline in tissue concentrations of arctic marine and terrestrial mammals (Muir et al. 1999, Muir & Norstrom 2000). Scattered reports also underline a general decrease in OCs for various species in different regions of the world: cod, flounder, mussels, and shrimp (Roose et al. 1998), foxes (Georgii et al. 1994), freshwater fishes (Schmitt et al. 1999), and herring gulls (Hebert et al. 1994, Ryckman et al. 1994). In environmentally exposed humans, almost all studies addressing the question of temporal trend of OCs have found a decreasing tendency (Dallaire et al. 2002, Harris et al. 1999, He et al. 2001, Noren 1993, Noren & Meironyte 2000, Schade & Heinzow 1998, Waliszewski et al. 1998). In these studies, the time required to halve the mean contaminant concentrations in the population ($t_{1/2}$) for PCB and DDT/DDE ranged from 4 to 7 years, except for PCBs in breast milk of Swedish women, which had a $t_{1/2}$ of 14 years (Noren & Meironyte 2000). These results are slightly lower but remain similar to what we observed in the present study ($t_{1/2}$ = 7.8 years and 7.1 years for PCBs and DDE, respectively). The generalized downward tendency of OC concentrations observed in wildlife and human tissues throughout the world strongly suggests that the environmental contaminant burden is steadily declining and that this tendency can be observed in all levels of the food chain. We believe that most of the decrease of OC concentrations observed in this study can be attributed to descending concentrations in the traditional food items of the inuit diet.

Sources of mercury and lead are diverse and are from both natural and anthropogenic origins. They are taken up by Arctic biota and depend on both local human activities and long-range transport (Dietz et al. 1998). Mercury and its organic form, methylmercury, bioaccumulate in the food chain reaching highest level in the meat of sea mammals. Again,

it is mainly through their traditional diet that inuit are exposed to mercury (Bjerregaard & Hansen 2000, Dewailly et al. 2001, Johansen et al. 2000, Muckle et al. 2001b). Recent data on temporal trends of mercury burden in wild animal are very limited. Wagemann et al. (1996) have noted an increase in mercury concentrations in beluga, ringed seal, and narwhal of the Canadian arctic between 1981 and 1994. In environmentally exposed humans, no statistically significant trends were noted in hair in a population of the Seychelles Islands between 1986 and 1989 (Cernichiari et al. 1995). Because no data on mercury temporal trends in arctic wildlife since 1994 are available, it is hazardous to speculate on the cause of the variations observed in this study. In contrast to OCs, our results for mercury do not support an exponential decrease. The concentrations were constant across the years, except for markedly lower levels in 1998 and 2000. When we used n-3 fatty acids in cord blood as a surrogate of maternal fish consumption, we observed a slight decrease that was not statistically significant and was not related to the mercury concentration. We also searched for relation between mercury concentration and the numbers of beluga caught in each village between 1996 and 2001, but no relation was found (Department of Fisheries and Oceans 2002). A thorough dietary survey would be necessary in order to elucidate the cause of the variation observed. Other studies with longer follow-up would clarify whether the lower levels observed in 1998 and 2002 were due to chance or were signs of a new temporal trend of mercury.

The sources of exposure to lead are less clear. Biomagnification of lead in the food chain does not play such an important role (Dietz et al. 1998, Muir et al. 1992). Rather, a recent study in Nunavik concluded that an important part of lead exposure could be from lead shots used for hunting (Levesque et al. 2003). Many reports have addressed the question of temporal trends of lead in human blood. Most authors have noted a strong decrease since the 1980's, mainly because of the switch from leaded to unleaded gasoline (Benes et al. 2001, Ducoffre et al. 1990, Kalina et al. 1999, Rothenberg et al. 2000, Wietlisbach et al. 1995). Few data are available on lead trends in tissues of arctic wildlife species. However, environmental lead contamination is low in the Arctic biota and does not seem to be the primary source of exposure for inuit population (Levesque et al. 2003). Unlike OCs and mercury, the observed decrease in lead concentration seems to be caused by the recent ban of lead shot combined with an information campaign on the potential health effects of lead

exposure due to lead shots. Our data strongly support this hypothesis: Lead concentration fell markedly in 1999, when lead shots were banned (0.12 µmol/L after the ban vs. 0.20 µmol/L before the ban, $p < 0.0001$). In fact, the fit of the statistical model is better when lead geometric mean levels before and after the ban are compared (in contrast to the exponential models used throughout this study for the other contaminants).

The present analysis was based on the merging of results from two different studies. We did not collect information from mothers refusing to participate to either study, so we cannot ascertain whether a selection bias was introduced in our results. Shortage of staff for cord blood sampling during delivery may have also introduced such a bias because participants for whom cord blood was available had slightly higher mean levels of OCs in their blood (maternal blood sample). The trends may therefore be underestimated. Finally, women with complicated or at risk deliveries were usually transferred to other hospitals. Our results therefore overrepresent healthier and uncomplicated pregnancies and deliveries.

Since the 1970's, many restrictions and regulations have helped to drastically reduce the input of POPs and heavy metals in the environment, and exposure through food contamination decreased accordingly. In our study population, we believe that POP decline is caused mainly by a diminution of food contamination and, to a lesser extent, dietary changes. It was well beyond to scope of this study to try to determine the specific contribution of these two factors to the observed decrease. Although questions remain as to the exact causes of the decline, it is encouraging to observe such an improvement in prenatal exposure for this highly exposed population. International efforts to further reduce the environmental input should be continued.

Acknowledgments

We are grateful to the Nunavik population for their participation in this research. We are indebted to Germain Lebel for management of the exposure data, and to Edna Lachance and Carole Vézina for their involvement in the data collection of the biological samples and medical information. This study was made possible by grant number R01-ES07902 from the National Institute of Environmental Health and Sciences/NIH, and grants from Indian and Northern Affairs Canada (Northern Contaminants Program), Health Canada, Hydro-

Québec (Environmental Child Health Initiative), and Nunavik Regional Board of Health and Social Services (Community Health Research Subsidy Program). F. Dallaire is supported by the Canadian Institutes of Health Research.

Chapitre 4 – Portrait de l'incidence d'infections aiguës chez les enfants du Nunavik de la naissance à 5 ans.

Dallaire F., Dewailly E., Vézina C., Bruneau S., et Ayotte P. 2006. Portrait of outpatient visits and hospitalizations for acute infections in Nunavik preschool children, *Canadian Journal of Public Health*, sous presse.

Résumé

Objectif: Les enfants inuits de la plupart des pays nordiques ont un haut taux d'incidence de maladies infectieuses. L'objectif de cette étude était d'évaluer le taux d'incidence d'infections aiguës d'enfants inuits du Nunavik d'âge préscolaire. **Méthodologie**: Les dossiers médicaux de 354 enfants ont été revus pour une période couvrant les cinq premières années de vie. Toutes les consultations médicales conduisant à un diagnostic d'infection aiguë et toutes les hospitalisations pour une infection aiguë ont été notées. **Résultats**: Le taux d'incidence d'otite moyenne aiguë était de 2314, 2300 et 732 événements par 1000 enfants-années pour les enfants âgés de 0-11 mois, 12-23 mois, et 2-5 ans respectivement. Le taux d'incidence d'infections des voies respiratoires inférieures était de 1385, 930 et 328 événements par 1000 enfants-années pour les enfants âgés de 0-11 mois, 12-23 mois, et 2-5 ans respectivement. Le taux d'incidence d'hospitalisation pour une pneumonie était de 198, 119 et 31 événements par 1000 enfants-années pour les enfants âgés de 0-11 mois, 12-23 mois, et 2-5 ans respectivement. **Conclusion**: Les résultats montrent que les enfants inuits du Nunavik avaient un taux d'incidence d'otites moyennes et d'infections des voies respiratoires inférieures supérieur à celui d'enfants de populations nord-américaines caucasiennes. Comparativement à d'autres populations canadiennes, les hospitalisations étaient presque 10 fois plus fréquentes au Nunavik.

Abstract

Objective: Inuit children for around the world are burdened by a high rate of infectious diseases. The objective of this study was to evaluate the incidence rate of infections in Inuit preschool children for Nunavik (Northern Québec). **Methods**: The medical chart of 354

children was reviewed for the first five years of life. All outpatient visits that led to a diagnosis of acute infection and all admission for acute infections were recorded. **Results**: Rates of outpatient visits for acute otitis media (AOM) were 2314, 2300, and 732 events/1000 children-year for children 0-11 months, 12-23 months, and 2-5 years, respectively. Rates of outpatient visits for lower respiratory tract infections (LRTIs) were 1385, 930, and 328 events/1000 children-year for children 0-11 months, 12-23 months, and 2-5 years, respectively. Rates of hospitalization for pneumonia were 198, 119, and 31 events/1000 children-year for children 0-11 months, 12-23 months, and 2-5 years, respectively. **Conclusion**: Compared to non-native North-American populations, Inuit children from Nunavik had higher rates of AOM and LRTIs. Admission for LRTIs are up to 10 times more frequent in Nunavik compared to other Canadian population.

Introduction

Acute infectious disease incidence has consistently been reported to be high in children of Inuit communities throughout the world (Banerji et al. 2001, Butler et al. 1999, Holman et al. 2001, Koch et al. 2002, Ling et al. 1969, Wainwright 1996). In Northern Canada, Alaska and Greenland, Inuit children have the highest prevalence of otitis media ever reported (Bluestone 1998). In Alaska, native children have higher rates of pneumococcal infections (Davidson et al. 1994, Wainwright 1996), respiratory syncytial virus infections (Karron et al. 1999, Lowther et al. 2000, Wainwright 1996), and hospitalization for respiratory infections (Holman et al. 2001), compared to Caucasian children. High hospitalization rates for lower respiratory tract infections (LRTIs) in Inuit children have also been observed in the Baffin region in Canada (Banerji 2001, Banerji et al. 2001) and Greenland (Koch et al. 2002).

The Nunavik region is located in the northernmost part of the province of Quebec. Around 9600 Inuit inhabit 14 communities spread out on the coast line of the Hudson Bay, the Hudson Strait, and the Ungava Bay. Similar to Inuit from other regions, children from Nunavik are burdened by a high incidence of acute infections. A study on ear problems in 800 Inuit children from 11 communities of Nunavik reported that 85% of them had ear problems (perforation, scarring, or active suppuration), with a prevalence of hearing deficit of 40% (Thérien 1988). In a recent report by Pageau and colleagues (2003), it was shown

that the rate of hospitalization for respiratory infections was 5 times higher in Inuit infants from Nunavik compared to that of infants from Southern Québec.

Our research group is studying the impact of food-chain contaminants on the immune system of Inuit children. During the last 10 years, we have assessed infection incidence and prevalence in children from Nunavik in order to evaluate their association with prenatal exposure to contaminants. By doing this, we have gathered an important body of data on infection incidence rates in these children. Our earlier studies underlined a considerably high incidence of acute infections. For example, in Inuit infants born in 1989-1990, we observed that the risk of having at least one episode of pulmonary infection was 59.3% during the first year of life (Dewailly et al. 2000). In infants < 3 months of age, 18.5% of infants had at least one episode of bronchitis or bronchiolitis, and 6.2% of them had at least one episode of pneumonia (Dewailly et al. 2000). In a subsequent cohort of 199 Inuit infants born between 1995 and 2001, we observed mean infection incidence rates of 2.4 episodes of acute otitis media (AOM) per infant-year, and of 1.7 episodes of LRTIs per infant-years, during the first year of life (Dallaire et al. 2004a).

Because little is known on acute infection incidence rates in Nunavik, and because we think that the observed incidence rates were high enough to deserve health workers attention, we constituted a third cohort of 354 preschool children for whom we reviewed the medical chart for a period encompassing their first five years of life. These children were born to mothers who had previously participated to a monitoring of umbilical cord blood contaminant concentrations between 1993 and 1996 (Dewailly et al. 1998a). The information collected has allowed us to draw a complete portrait of outpatient visits and hospitalization for acute infections for these children. In this study, we report the incidence rates of outpatient visits and hospitalizations for the most common infections in preschool children of Nunavik. Risk-factors specific to this population, such as vitamin A deficiency and exposure to food-chain contaminants, are also being investigated in the same cohort and results will be published separately.

Materials and methods

Study population

Between 1993 and 1996, 491 unselected pregnant women from the 14 Inuit communities of Nunavik were enrolled in a study on prenatal exposure to food-chain contaminants (Dewailly et al. 1998a). The women were invited to participate at their arrival at one of the two health centers in Nunavik for delivery (Puvirnituq and Kuujjuaq, see figure 3.1). Women giving birth elsewhere were not included. An umbilical cord blood sample was taken and an interview was conducted with the mothers few months after delivery. Children born to these mothers were the targeted participants for the current study.

Medical chart review

We attempted review the medical charts of all the children included in the above-mentioned study. We made a list of all the medical chart numbers available in our database and worked with the staff of the communities' health centers to locate the charts. We then had the charts copied and sent to our research center for review. Five second- and third-year trained medical students reviewed the charts using a standardized questionnaire. The questionnaire was focused on acute infections, but most health problems were recorded. For every diagnosis of infection noted in the charts, we recorded the date of diagnosis, whether antibiotics were prescribed, and whether the child was hospitalized. For each infection, we also attributed a code corresponding to the International Classification of Primary Care, 2nd edition (ICPC-2) of the World Organization of National Colleges, Academies and Academic Associations of General Practitioners (WONCA 1998). For some infection types, we grouped together infections of similar natures. For example, pneumonia, bronchitis and bronchiolitis were all included in the LRTIs category. Table 4.1 details which infections are included in these categories. When the child was hospitalized, we recorded the main reason of hospitalization, whether concurrent illnesses were present, the date of hospitalization, the length of stay, and whether the child was transferred to another hospital.

Table 4.1
Description of infection classification and ICPC-2 codes

Category	Codes ICPC-2	Infections included
Upper respiratory tract infections (URTIs)	R72	Streptococcal pharyngitis and tonsillitis.
	R74	Acute upper respiratory tract infection, acute rhinitis, head cold, nasopharyngitis, pharyngitis, and coryza.
	R75	Sinusitis
	R76	Tonsillitis
	R77	Laryngitis, tracheitis, and croup
	R80	Influenza
Lower respiratory tract infections (LRTIs)	R78	Acute bronchitis or bronchiolites, acute lower respiratory infection NOS, chest infection NOS, laryngotracheobronchitis, and tracheobronchitis.
	R81	Bacterial and viral pneumonia, bronchopneumonia, influenzal pneumonia, Legionnaire's disease, and pneumonitis.
Gastro-intestinal infections (GIIs)	D70	Gastrointestinal infection or dysentery with specified organism.
	D73	Diarrhea or vomiting presumed to be infective, dysentery NOS, and gastric flu.
Cutaneous infections	S71	Herpes simplex
	S72	Scabies and other acariases
	S84	Impetigo
	S76	Other skin infections NOS
Other viral diseases	A76	Viral exanthem
	A77	Other viral disease NOS

NOS = Not otherwise specified; ICPC-2 = International classification of primary care, 2nd edition

For every health problem identified, we always trusted the diagnosis of the attending physician. When two physicians disagreed, we only recorded the last diagnosis made. In some Inuit communities, nurses are trained to identify and treat benign infections, especially otitis media and upper respiratory tract infections. When the child was not seen by a physician, we recorded the diagnosis of the nurse. Only new illnesses were recorded. We excluded follow-up visits and consultations for unresolved infections previously recorded. We did not gather information on infectious episodes for which parents decided not to seek medical attention.

Statistical analyses

For each type of infections, we computed the incidence of new episodes for each participants. Each infectious episode was considered independent from all other episodes, including the ones of the same nature. However, we excluded episodes that were diagnosed within 15 days of a previous episode affecting the same anatomic site. We computed mean

incidence rates using the expression [(total number of episodes ÷ total duration of follow-up) × 1000]. Since a second infection affecting the same anatomic site was excluded if it occurred less than 15 days after a first infection, these fifteen days were considered not "at risk" and were excluded for the follow-up duration. Hence, incidence rates are presented as the number of episodes per 1000 child-years at risk. We used SPSS Data Entry Builder 2.0 for data entry (Chicago, Illinois, United States) and SAS 8.02 (Cary, NC, United States) for database management and statistical analyses.

Results

Participants

Four hundred ninety-two mothers originally participated in the study on contaminants in umbilical cord blood. There was not enough information for us to trace the charts for 37 (7.5%) children and it was impossible to get 45 (9.1%) charts for various logistical reasons. Of the 410 charts available, 28 (6.8%) were incomplete, 17 (4.1%) families moved out of Nunavik during follow-up, 8 (2.0%) children died and 3 (0.7%) children were excluded because they suffered from a serious chronic disease. The final analyses included the 354 remaining children. Table 4.2 shows the characteristics of these children and their mothers.

Table 4.2
Characteristics of participants

Characteristics	Mean value (STD) or percentage (n = 354)
Children	
Sex (male)	48.6%
Given to adoption	23.8%
Year of birth	
1994	37.3%
1995	32.5%
1996	30.2%
Hospital of delivery	
Puvirnituq	50.0%
Kuujjuaq	50.0%
Gestational age	
Mean gestational age	39.0 weeks
Premature (< 37 weeks)	5.1%
Village of residence	
Kuujjuaraapik	4.0%
Umiujak	1.1%
Inukjuaq	11.7%
Puvirnituq	16.8%
Akulivik	5.4%
Ivujivik	2.9%
Salluit	8.0%
Kangiqsujuaq	6.0%
Quaqtaq	6.6%
Kangirsuk	5.4%
Tasiujaq	0.9%
Kuujjuaq	21.1%
Kangiqsualujjuaq	10.3%
Birthweight (g)	3486 g
Length (cm)	51.4 cm
Mothers	
Age	23.8 years
Parity	2.08

Infection incidence rate

The review of the charts identified 8031 consultations leading to a diagnosis of a newly developing acute infection before the age of 5 years. Two hundred forty-six episodes were excluded because they were diagnosed within fifteen days of a previous episode affecting the same anatomic site. Table 4.3 shows the incidence of the 17 most frequent infections. In children less than 12 months old, AOM had the highest incidence (2314 episodes per 1000

child-years), followed by URTIs (2126 episodes per 1000 child-years) and LRTIs (1385 episodes per 1000 child-years). The other most frequent infections, in order of decreasing incidence rate, were gastro-intestinal infections, cutaneous infections, conjunctivitis, chickenpox, other systemic viral diseases, otitis externa, and urinary tract infections (UTIs).

Table 4.3
Incidence rate of acute infections

Infection	**Incidence rate** (events per 1000 children-year)		
	0 to 11 months old	**12 to 23 months old**	**2 to 4 years old**
Acute otitis media	2313.9 (2073.5 – 2554.3)	2300.4 (2043.5 – 2557.3)	732.3 (643.0 – 821.6)
Upper respiratory tract infections	2125.7 (1914.8 – 2336.3)	1572.9 (1402.3 – 1743.5)	930.7 (817.2 – 1044.3)
Lower respiratory tract infections	1385.4 (1197.9 – 1572.8)	929.7 (794.9 – 1064.5)	328.3 (279.9 – 376.7)
Gastro-intestinal infections	404.9 (332.5 – 477.4)	326.4 (257.7 – 395.0)	179.3 (149.9 – 209.7)
Cutaneous infections	309.0 (239.0 – 378.9)	513.9 (424.7 – 602.2)	229.1 (193.0 – 265.2)
Conjunctivitis	210.9 (150.8 – 270.9)	116.4 (81.0 – 151.8)	89.8 (70.1 – 109.5)
Chicken pox	133.5 (79.5 – 147.5)	102.1 (69.7 – 134.6)	80.3 (65.2 – 95.3)
Viral diseases	68.0 (39.7 – 96.2)	76.5 (44.4 – 108.6)	39.6 (26.5 – 52.7)
Otitis externa	39.6 (17.1 – 62.0)	107.8 (65.8 – 149.8)	28.3 (17.8 – 38.8)
Urinary tract infections	36.8 (15.0 – 58.6)	59.5 (29.1 – 89.8)	41.5 (27.4 – 55.6)
Meningitis	11.3 (0.0 – 22.6)	5.7 (-2.4 – 13.7)	0.9 (-0.9 – 2.8)
Blepharitis / style / chalazion	11.3 (0.0 – 22.6)	2.8 (-2.8 – 8.5)	4.7 (0.6 – 8.9)
Balanitis	8.5 (-1.3 – 18.3)	8.5 (-1.3 – 18.3)	6.6 (1.0 – 12.2)
Post traumatic skin infections	8.5 (-1.3 – 18.3)	5.7 (-2.4 – 13.9)	5.6 (1.1 – 10.2)
Pyelonephritis / pyelitis	5.7 (-2.4 – 13.9)	2.8 (-2.8 – 8.5)	3.8 (-0.8 – 8.3)
Worms and parasites	0.0 (–)	2.8 (-2.8 – 8.5)	6.6 (-0.2 – 13.4)
Vaginitis and vulvitis	0.0 (–)	0.0 (–)	9.4 (3.0 – 15.8)

In children aged 12-23 months, the incidence rates were lower than children < 12 months old, except for cutaneous infections, systemic viral diseases, otitis externa, and UTIs. The most frequent infections were again AOM (2300 episodes per 1000 child-years), URTIs (1573 episodes per 1000 child-years), and LRTIs (930 episodes per 1000 child-years).

As expected, in children aged 2-5 years, the incidence rates were much lower than in younger children. Upper respiratory tract infections was the most frequent (931 episodes per 1000 child-years), followed by AOM (732 episodes per 1000 child-years), LRTIs (328 episodes per 1000 child-years), and cutaneous infections (229 episodes per 1000 child-years).

Figure 4.2 shows the incidence rates of AOM, URTIs, LRTIs, Cutaneous infections, gastro-intestinal infections, and conjunctivitis, according to the age of the children. All 6 infections peaked before 1 year of age. Acute otitis media had the highest incidence between 6 and 11 months while URTI plateaued during the first 9 months and decreased afterwards. Lower respiratory tract infections peaked before 6 months and also decreased steadily afterwards.

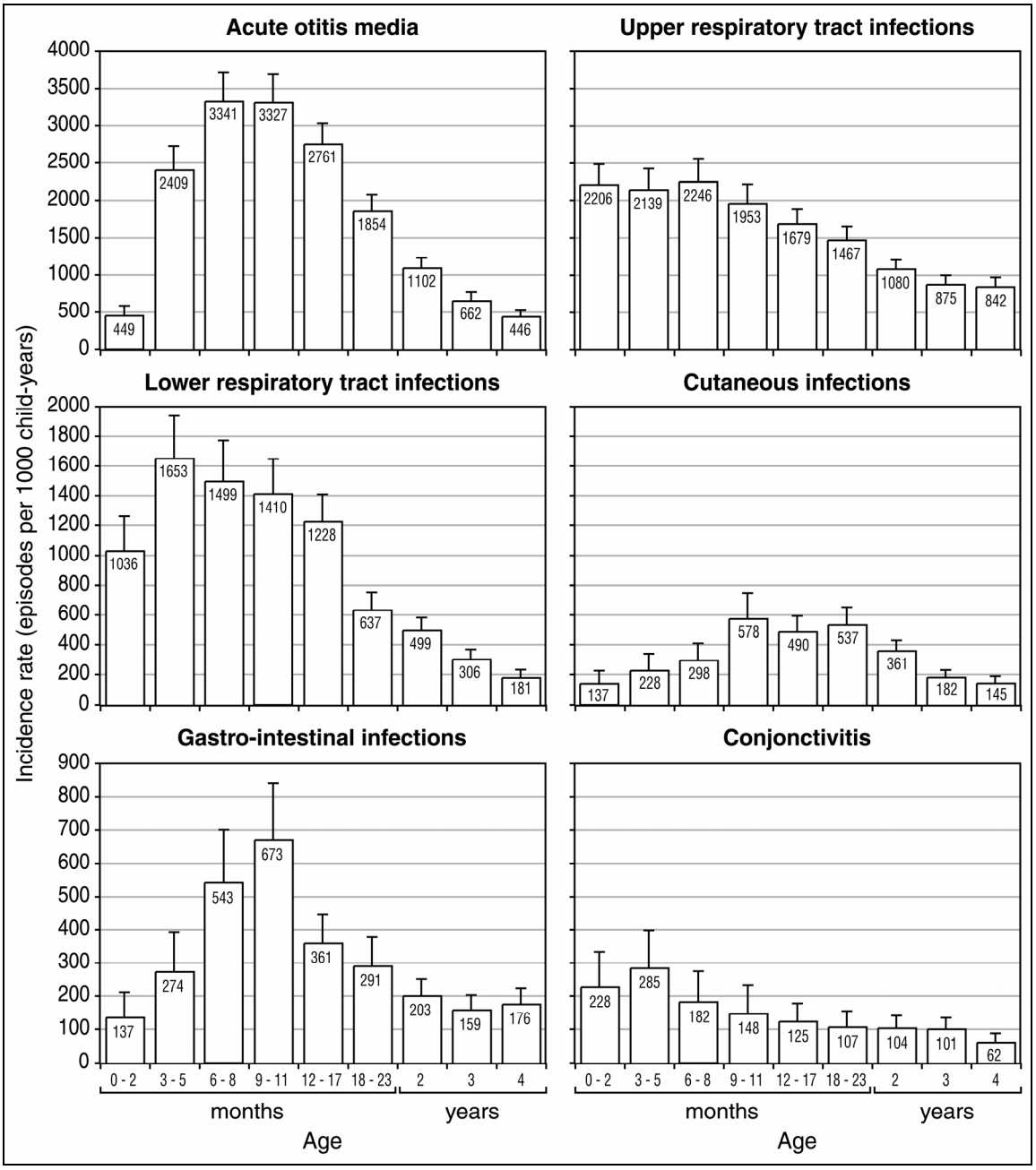

Figure 4.2 Incidence rates of outpatient visits for the most frequent acute infections in Inuit preschool children according to age.

Hospitalizations

We identified 302 hospitalizations related to infectious diseases during the complete follow-up. Table 4.4 presents the incidence rate of the most frequent hospitalization for infectious diseases. Hospitalization for infections were far more frequent before 1 year of

age. In children aged < 1 year old, pneumonia was the most frequent (197.7 hospitalizations per 1000 child-years), followed by bronchitis/bronchiolitis (107.3 hospitalizations per 1000 child-years), URTIs (28.3 hospitalizations per 1000 child-years), and UTIs (16.9 hospitalizations per 1000 child-years).

Table 4.4
Incidence rate of hospitalizations for acute infections

Principal diagnosis on admission	**Incidence rate** (events per 1000 children-year)		
	0 to 11 months old	**12 to 23 months old**	**2 to 4 years old**
Pneumonia	197.7 (144.9 – 250.6)	118.6 (77.4 – 159.9)	30.1 (16.6 – 43.6)
Bronchitis / bronchiolitis	107.3 (68.0 – 146.7)	22.6 (5.2 – 40.0)	4.7 (-0.2 – 9.6)
Upper respiratory tract infections [a]	28.3 (7.6 – 48.9)	0.0 (–)	0.9 (-0.9 – 2.8)
Urinary tract infections	16.9 (3.4 – 30.5)	22.6 (5.2 – 40.0)	1.9 (-0.7 – 4.5)
Fever NOS	14.1(1.8 – 26.5)	2.8 (-2.7 – 8.4)	0.9 (-0.9 – 2.8)
Acute otitis media	11.3 (0.2 – 22.4)	2.8 (-2.7 – 8.4)	0.9 (-0.9 – 2.8)
Cellulitis	8.5 (-1.1 – 18.1)	14.1 (-0.5 – 28.8)	7.5 (1.2 – 13.9)
Meningitis	8.5 (-1.1 – 18.1)	5.6 (-2.2 – 13.5)	0.9 (-0.9 – 2.8)
Pyelonephritis / pyelitis	5.6 (-2.2 – 13.5)	2.8 (-2.7 – 8.4)	2.8 (-0.4 – 6.0)
Laryngitis / tracheitis	2.8 (-2.7 – 8.4)	8.5 (-1.1 – 18.1)	2.8 (-0.4 – 6.0)

[a] For hospitalization, the upper respiratory tract infections category did not include streptococcal throats, sinusitis, tonsillitis, laryngitis, tracheitis, croup, and influenza (ICPC-2 codes R72, R75-77, and R80). These infections were analyzed separately.

In older children, pneumonia was responsible for most hospitalizations with an incidence rate of 118.6 hospitalizations per 1000 child-years for children between 1 and 2 years old, and 31.1 hospitalizations per 1000 child-years for children between 2 to 5 years-old. In general, the mean duration of hospitalization was less than 6 days, except for meningitis, which had a mean hospital stay of 13 days.

Discussion

The aim of this study was to document the incidence rates of outpatient visits and hospitalizations for acute infections in preschool Nunavik children. To our knowledge, this is the first study documenting the incidence of acute infections in this population, both on an outpatient and an inpatient basis. Through a thorough review of the medical charts of 354 children from 13 communities of Nunavik, we found a high incidence rate of acute ear and respiratory infections. Furthermore, we observed that a great proportion of the children had to be hospitalized, especially for LRTIs. The children included in this study were all born in Nunavik. Mothers giving birth elsewhere were excluded. Because women with at-

risk pregnancies often give birth in a tertiary center in Montreal or Quebec City, and because these women were excluded from our analysis, it can be expected that a bias towards healthier pregnancies is present in our results.

In this study, we used a review of the medical charts to evaluate disease frequency. There is only one health center in each community included in this study. Participants almost always go to that health center when they seek medical attention, and copies of consultations done elsewhere are routinely requested to complete medical charts. We are therefore confident that we have reviewed a majority of outpatient visits sought by the participants. However, we did not attempt to verify every diagnosis, nor did we try to inquire about infections for which medical attention was not sought by the parents. It is therefore important to keep in mind that the incidence rates reported here are underestimated, especially for benign infection. Although the magnitude of this underestimation is difficult to evaluate, we can assume that it will be related to both the severity of the symptoms and the parent's perception of the disease. It has been previously shown by Saunders and colleagues (2003) that respiratory symptoms perceived as severe, high fever, young age, and earache have the strongest influence on the parent's decision to seek medical attention. Although differences in culture and background exist between the middle-class urban population included in Saunders and colleague's study and the Inuit population included in our study, it is likely that these factors also influenced the incidence rates reported here.

Our literature review has allowed us to identify relatively few recent published studies addressing the question of outpatient visits for acute infection in children. Table 4.5 summarizes and compares the incidence rates previously published with that observed in the present study. Our results showed that AOM was the most frequently diagnosed health problem. Most studies addressing the question of ear infections in Inuit populations have focused on chronic otitis media, scarring and hearing deficit (Baxter 1999, Bluestone 1998, Julien et al. 1987, Thérien 1988). From these studies, it is well recognized that Inuit children are burdened by ear infections, but few recent articles have been published on the incidence of AOM. Our results show that the incidence of outpatient visits for AOM in Inuit children from Nunavik was higher than that of aboriginal children from a remote community of Northern Ontario (Harris et al. 1998). It was also higher than that of US

children, but similar to what was observed in Alaska and Saskatchewan (Curns et al. 2002, Wang et al. 1999). It thus seems that the burden of ear infections in Nunavik is comparable to other aboriginal children from North America. However, because ear infections is such a well-recognized problem in Nunavik, we found it surprising that non-native children from Saskatchewan had rates similar to native and Inuit children. With the data available, we do not know whether this was due to similar incidence rates or to differences in parents attitudes and perceptions towards medical visits for ear symptoms.

Table 4.5
Comparison between incidence rates observed in this study and in other recent studies for outpatient visit rates

Infection	Population	Age group	Rates of outpatient visits (event / child-year)			Reference
			Cited study	Present study [a]	Rate ratio (present / cited)	
AOM	Isolated aboriginal community, Ontario, Canada	<1 y	1.62	2.11	1.30	Harris et al. (1998)
AOM	Isolated aboriginal community, Ontario, Canada	1-4 y	0.64	1.06	1.66	Harris et al. (1998)
Otitis related problems	Native children, USA	< 1 y	3.18	2.42	0.76	Curns et al. (2002)
Otitis related problems	Native children, USA	1-4 y	1.07	1.32	1.23	Curns et al. (2002)
Otitis related problems	Natives from Alaska, USA	0-4 y	1.58	1.54	0.97	Curns et al. (2002)
Otitis related problems	General population, USA	0-4 y	0.74	1.54	2.08	Curns et al. (2002)
AOM	Non-native, Saskatchewan, Canada	0-4 y	1.2	1.27	1.06	Wang et al. (1999)
URTIs	Isolated aboriginal community, Ontario, Canada	<1 y	2.17	1.95	0.90	Harris et al. (1998)
URTIs	Isolated aboriginal community, Ontario, Canada	1-4 y	0.78	1.04	1.33	Harris et al. (1998)
URTIs	Non-native, Saskatchewan, Canada	0-4 y	1.86	1.22	0.66	Wang et al. (1999)
URTIs	Inuit, Greenland	0-2 y	1.86	1.85	0.99	Koch et al. (2003, 2002)
LRTIs	Isolated aboriginal community, Ontario, Canada	<1 y	0.20	1.31	6.55	Harris et al. (1998)
LRTIs	Isolated aboriginal community, Ontario, Canada	1-4 y	0.065	0.47	7.23	Harris et al. (1998)
LRTIs	Non-native, Saskatchewan, Canada	0-4 y	0.33	0.64	1.94	Wang et al. (1999)
LRTIs	Inuit, Greenland	0-2 y	1.78	1.16	0.65	Koch et al. (2003, 2002)

AOM = acute otitis media; URTIs = upper respiratory tract infections; LRTIs = lower respiratory tract infections.
[a] Whenever possible, the rates for the present study were calculated in order to reflect the method used by the cited studies.

We found that upper and lower respiratory tract infections were responsible for almost 30% of all outpatient visits for acute infections. Our results show that URTIs were diagnosed as often as in other native populations, but less frequently than in non-native from

Saskatchewan (Harris et al. 1998, Koch et al. 2003, Koch et al. 2002, Wang et al. 1999). However, LRTIs were far more frequent in Nunavik children, exception made of Inuit from Greenland, who had the highest rates reported (Harris et al. 1998, Koch et al. 2003, Koch et al. 2002, Wang et al. 1999). There may be great variability among parents, physicians and investigators, on the perception and interpretation of respiratory symptoms, especially for the upper respiratory tract. It is therefore hazardous to infer from outpatient visits that differences between rates are solely due to difference in the incidence of development of URTIs. On the other hand, LRTIs are usually accompanied by more serious symptoms and it is likely that the proportion of children with an undiagnosed acute infection of the lower respiratory tract will be smaller. According to our results, LRTIs infections in Nunavik are more frequent than in most other populations. So far, public health initiatives in Nunavik have been focused on ear problems. We believed that no effort should be spared to address the problem of LRTIs as well.

In the present study, the rate of hospitalization was extremely high, as 56.8% of the children were hospitalized at least once, and more than 16% were hospitalized three times or more before they reached the age of five years. Table 4.6 shows the comparison between the rates observed and those recently published in the scientific literature. The hospitalization rate observed in the present study was lower than that previously found in the same population (Pageau et al. 2003). It was however much higher compared to other native and non-native populations (Brownell et al. 2002, Holman et al. 2001, Kozyrskyj & Hildes-Ripstein 2002, Pelletier 1999, Wang et al. 1999). In children aged < 1 year, the rate of hospitalization for LRTIs alone is higher in Nunavik than the rate of hospitalization for all causes in Manitoba and Southern Quebec (Brownell et al. 2002, Pelletier 1999). It is also twice that of low-income neighborhoods in Manitoba, and up to 20 times that of the wealthiest area of Winnipeg (Kozyrskyj & Hildes-Ripstein 2002). These observations show that the high incidence of LRTIs translates into an elevated number of hospitalizations. Because the difference between Nunavik children and other population is greater for inpatient events compared to outpatient visits, we conclude that not only these children are burden by a high rate of LRTIs, but that these infections seem more severe and lead to an even greater rate of hospitalization. This could also be due, in part, by a greater access to inpatient treatments in Northern communities.

Table 4.6
Comparison between incidence rates observed in this study and in other recent studies for hospitalizations

Hospitalization	Population	age group	Rates of hospitalization (event / child-year)			Reference
			Cited study	Present study [a]	Rate ratio (present / cited)	
All causes	Inuit, Nunavik (Québec, Canada) 1991-1996	< 1 y	0.68	0.571	0.84	Pageau et al. (2003)
All causes	Inuit, Nunavik (Québec, Canada) 1996-2001	< 1 y	0.88	0.571	0.65	Pageau et al. (2003)
All causes	Southern Quebec, Canada, 1991-1996	< 1 y	0.23	0.571	2.48	Pelletier (1999)
All causes	Southern Quebec, Canada, 1996-2001	< 1 y	0.26	0.571	2.20	Pelletier (1999)
All causes	Manitoba, Canada	< 1 y	0.22	0.571	2.60	Brownell et al. (2002)
All causes	Manitoba, Canada	1-4 y	0.052	0.177	3.40	Brownell et al. (2002)
All infectious diseases	Native, USA, 1994	<1	0.156	0.401	2.57	Holman et al. (2001)
All infectious diseases	Native, USA, 1994	1-4 y	0.0198	0.090	4.55	Holman et al. (2001)
LRTIs	Inuit, Nunavik (Québec, Canada) 1991-1996	<1 y	0.332	0.305	0.92	Pageau et al. (2003)
	Inuit, Nunavik (Québec, Canada) 1996-2001	< 1 y	0.406	0.305	0.75	Pageau et al. (2003)
LRTIs	Low-income neigborhood, Manitoba, Canada	< 1 y	0.150	0.305	2.03	Kozyrskyj et al. (2002)
Pneumonia	Non-native, Saskatchewan, Canada	0-4 y	0.0070	0.0693	9.90	Wang et al. (1999)

LRTIs = lower respiratory tract infections.
[a] Whenever possible, the rates for the present study were calculated in order to reflect the method used by the cited studies.

In this study, we drew a complete portrait of the incidence of infections leading to an outpatient visits, and to admission in children of Nunavik. We believe that this information is important for public health purposes, especially to identify acute pathologies that represent an important burden for the children, the parents, the communities, and the health care system. It is beyond the scope of this paper to find explanations for the elevated rates observed. Compared to the general population of Canada, some important risk factors for infection, such as tobacco exposure, crowding, and poor maternal education are more prevalent in Nunavik (Pageau et al. 2003). Preterm births, complicated deliveries and vaccination coverage do not seem, however, to be problematical in Nunavik (Pageau et al. 2003). Other research avenues, such as food-chain contaminants exposure and vitamin A

deficiency, are currently being investigated, but it is unlikely these aspects are alone responsible for the elevated rates observed. Further studies are needed to better grasp this likely multi-factorial situation in order to plan well-needed public health interventions.

Acknowledgments

We are grateful to the Nunavik population for their participation in this research. We are indebted to Germain Lebel for management of the initial database. We thank Marie-Lise Mercier, Mélanie Gaudreault, Catherine Lalonde, Élisabeth Leblanc, and Valérie Marchand for medical charts review, and Patsy Tulugak and Mary Nulukie for help with charts retrieval and copying.

Chapitre 5 – Infections aiguës et exposition environnementale aux organochlorés chez les bébés inuits du Nunavik

Dallaire F., Dewailly E., Muckle G., Vézina C., Jacobson S.W., Jacobson J.L. et Ayotte P., 2004. Acute infections and environmental exposure to organochlorines in Inuit infants from Nunavik. *Environ Health Perspect* 112(14) : 1359-65.

Résumé

La population inuite du Canada est exposée à des composés organochlorés immunotoxiques principalement par le biais de sa consommation de poisson et de mammifères marins. Dans cette étude, l'effet d'une exposition périnatale aux biphenyls polychlorés (BPC) et aux dichlorodiphenyl dichloroethylène (DDE) sur l'incidence d'infection aiguës chez les bébés inuits a été investigué. Les dossiers médicaux d'une cohorte de 199 bébés inuits ont été revus pour une période couvrant la première année de vie. Les taux d'incidence d'infections des voies respiratoires supérieures et inférieures, d'otites moyennes et d'infections gastro-intestinales ont été évalués. Un échantillon de plasma a été prélevé chez les mères pendant le travail obstétrical et chez les enfants à 7 mois. Les BPC et le DDE y ont été dosés. Pour les 6 premiers mois de suivi, comparés aux enfants du premier quartile d'exposition de BPC (les moins exposés), les rapport de taux ajustés des enfants des quartiles d'exposition supérieurs s'étendaient de 1,09 à 1,32 pour les infection respiratoires hautes, de 0,99 à 1,39 pour les otites moyennes, de 1,52 à 1,89 pour les infections gastro-intestinales et de 1,16 à 1,68 pour les infections des voies respiratoires inférieures. Lorsque toutes ces infections sont combinés, les rapport de taux s'étendaient de 1,17 à 1,27. L'association était semblable pour l'exposition au DDE mais était plus faible pour le suivi de 12 mois. Globalement, la plupart des rapport de taux étaient supérieurs à 1,0 mais peu étaient statistiquement significatifs ($p < 0.05$). Aucune association n'a été notée pour l'exposition postnatale. Ces résultats montrent une association possible entre l'exposition prénatale aux organochlorés et l'incidence d'infection aiguës précoces chez les bébés inuits du Nunavik.

Abstract

The Inuit population of Nunavik (Canada) is exposed to immunotoxic organochlorines (OCs) mainly through the consumption of fish and marine mammal fat. The effect of perinatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethylene (DDE) on the incidence of acute infections in Inuit infants was investigated. The medical charts of a cohort of 199 Inuit infants were reviewed during the first 12 months of life. Incidence rates of upper and lower respiratory tract infections (URTI and LRTIs), otitis media, and gastrointestinal (GI) infections were evaluated. Maternal plasma during delivery and infant plasma at 7 months were sampled and assayed for PCBs and DDE. Compared to infants in the first quartile of exposure to PCBs (least exposed), adjusted rate ratios for infants in higher quartiles ranged between 1.09-1.32 for URTIs, 0.99-1.39 for otitis, 1.52-1.89 for GI infections, and 1.16-1.68 for LRTIs during the first 6 months of follow-up. For all infections combined, the rate ratios ranged from 1.17 to 1.27. The effect-size was similar for DDE exposure, but was lower for the full 12-month follow-up. Globally, most rate ratios were above 1.0, but few were statistically significant ($p < 0.05$). No association was found when postnatal exposure was considered. These results show a possible association between prenatal exposure to OCs and acute infections early in life in this Inuit population.

Introduction

Substantial information concerning the contamination of Northern marine food by organochlorines (OCs) is now available (Braune et al. 1999, Burkow & Kallenborn 2000, Muir et al. 1999). This family of compounds includes chlorinated pesticides [dichlorodiphenyl trichloroethane (DDT), dieldrin, mirex, and toxaphene] and industrial compounds [hexachlorobenzene (HCB) and polychlorinated biphenyls (PCBs)]. Several OCs are chemically stable. They are thus resistant to biodegradation and can accumulate in adipose tissue of living organisms. This leads to their biomagnification in the aquatic and terrestrial food chain, resulting in the highest levels in top predator species (Braune et al. 1999, Evans et al. 1991, Muir et al. 1999, Skaare et al. 2000). The manufacture of most OCs was halted in the 1970's when regulatory actions were adopted to limit their

production and use. Today, OCs are still released into the environment due to improper storage and ongoing use in certain parts of the world.

For cultural and economical reasons, carnivorous fish and marine mammals constitute an important part of the diet of the Inuit population of Nunavik (Northern Quebec, Canada). Their exposure to biomagnified substances, such as OCs, is thus proportionally high. Several studies have identified markedly higher concentrations of OCs in adult blood, umbilical cord blood, and breast milk of Nunavik inhabitants, compared to those of the Southern Québec population (Ayotte et al. 1997, Ayotte et al. 2003, Dewailly et al. 1993, Dewailly et al. 1998b, Dewailly et al. 1989, Muckle et al. 2001b, Muckle et al. 1998, Rhainds et al. 1999).

Exposure to most OCs produces a wide variety of immunotoxic effects in animals and humans. OCs that have a chemical structure similar to 2,3,7,8-tetrachlorodibenzo-*p*-dioxin, such as dioxin congeners and coplanar PCBs, are especially immunotoxic. Alterations of T-cell subsets, of serum IgA and IgM concentrations, of delayed-type hypersensitivity, and of complement function, have been documented in primates and human (Belles-Isles et al. 2002, Chang et al. 1982, Hoffman et al. 1986, Lu & Wu 1985, Neubert et al. 1992, Tryphonas et al. 1991a, Tryphonas et al. 1991b). The development of the immune system *in utero* and during infancy is particularly sensitive to immunotoxic agents. High exposure during early life could lead to permanent defects in the immune system and thus decrease resistance to infectious agents (Badesha et al. 1995).

The high incidence of acute infectious diseases in infants and children from Nunavik has been known for many years (Bruneau et al. 2001, Dufour 1988, Proulx 1988, Thérien 1988). In this context, we hypothesized that the incidence of infections among Inuit infants was related in part to the high maternal body burden of immunotoxic food-chain contaminants during pregnancy. In 2000, we published a first study on susceptibility to infection in Inuit infants recruited between 1989 and 1990 (Dewailly et al.). We found that the risk of acute otitis media and recurrent otitis media was positively associated with prenatal exposure to OCs. However, postnatal exposure was not considered, and some potential confounding factors could not be evaluated. In order to confirm the association observed, we investigated the association between exposure to OCs and the incidence rate

of acute infections during the first year of life in a second cohort of 199 Inuit infants recruited between 1995 and 2001.

Material and methods

Study population and recruitment

The Nunavik region is located north of the 55th parallel in the province of Québec (Canada) and is composed of 14 isolated villages scattered along the coasts of the Ungava Bay, the Hudson Strait and the Hudson Bay (Figure 3.1). The targeted participants for this study were Inuit infants born in Puvirnituq, Inukjuaq and Kuujjuarapik, the three largest Inuit communities on the Hudson Bay coast in Nunavik. The recruitment procedures have been described elsewhere (Muckle et al. 2001b). Briefly, between November 1995 and March 2001, we attempted to contact every pregnant woman after their first prenatal medical visit by phone or by the community radio (for those without telephone at home). Pregnant women were invited to meet with our research assistant, and women willing to participate were asked to sign an informed consent form. The study was part of a larger study focusing on environmental contaminants and neurobehavioral development. The study protocol was reviewed and approved by the Nunavik Health and Nutrition Committee and by the ethics committee of Laval University.

Data collection and biological sampling

In order to gather biological samples and information on confounding variables, we conducted four interviews: one at mid-pregnancy (pre-natal interview, median = 21 weeks of gestation) and three with the infant and the mother at 1, 6 and 11 months postpartum. We collected information on maternal age, breastfeeding duration, socioeconomic status of the caregiver (Hollingshead index), smoking habits during pregnancy, environmental tobacco exposure during the first year of life, number of children living with the participant, village of residence, and daycare attendance. Many other characteristics were also documented for the neurobehavioral arm of this cohort but were not included in this study.

We sampled maternal blood at delivery or, when it was impossible, as soon as possible after delivery (median = 2 days postpartum). We also obtained umbilical cord blood at delivery and infant blood at mid follow-up (median = 7.0 months of age). All blood

samples were immediately centrifuged and frozen at –80 °C. Frozen blood and plasma samples were sent to the Centre de Toxicologie (Institut National de Santé Publique du Québec, Québec City, Canada) every 3 to 6 months for contaminants and biochemical analyses. Finally, we extensively reviewed the medical charts of the mother and the infant for the pregnancy period and for the first year of life of the infant.

Determination of OCs

We determined the concentrations of *p,p′*-dichlorodiphenyl dichloroethylene (DDE) and of 14 PCBs congeners (International Union of Pure and Applied Chemistry numbers 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183 and 187) in plasma samples by high-resolution gas chromatography. OCs were extracted from plasma with ammonium sulfate : ethanol : hexane (1 : 1 : 3). The extracts were cleaned on florisil columns, taken to a final volume of 100 µL, and analyzed on an HP-5890 series II gas chromatograph equipped with dual-capillary columns and dual Ni-63 electron-capture detectors (Hewlett-Packard, Palo Alto, CA). We identified peaks by their relative retention times obtained on the two columns. Quality control procedures were described previously (Rhainds et al. 1999). Percent recovery ranged from 89% to 100%, and the detection limit was approximately 0.02 µg/L for all compounds. Coefficients of variation ($n = 20$, different days) ranged from 2.1% to 9.1%. The difference between the concentration of reference material and that found using the analytic method ranged from 10.9% to 3.8%. Because OCs are stored mainly in body fat, all contaminants results are expressed on a lipid basis.

Determination of blood lipids

We measured total cholesterol, free cholesterol and triglycerides in plasma samples by standard enzymatic procedures. Phospholipids concentrations were determined according to the enzymatic method of Takayama et al. (1977) using a commercial kit (Wako Pure Chemical Industries, Richmond, VA). We estimated the concentrations of total plasma lipids using the formula developed by Phillips et al. (1989).

Estimation of exposure using plasma concentrations

In this population, maternal OCs concentrations are highly correlated with cord plasma concentration (R = 0.94 for DDE and PCB congener 153). Due to logistic problems, we

were not able to collect cord blood samples for more than half of the participants. We, therefore, used the concentration of OCs in maternal plasma as an estimate of prenatal exposure to OCs. For six subjects, a cord blood sample was available but not a maternal blood sample. For these six subjects, we estimated maternal concentrations from the cord plasma results using linear regression. Postnatal exposure was estimated using plasma concentration of OCs in infant blood at 7 months of age. The concentration of OCs in blood is well correlated with that found in adipose tissues and it has been shown that either blood or adipose tissue concentrations are valid exposure measurements in epidemiological studies (Dewailly et al. 1994).

PCB congener 153 concentration (log-transformed) was used as a surrogate measure for the total PCB burden. PCB 153 is the most abundant PCB congener. Its concentration is strongly correlated with all the moderate-to-heavily chlorinated congeners and with most chlorinated pesticides (except *p,p´*-DDT). It has been shown to be a good marker of exposure to most organochlorines in the Arctic (Muckle et al. 2001b).

Medical chart review and infectious diseases incidence

Trained research nurses reviewed the medical charts of infants for the first 12 months of life using a standardized questionnaire. For every diagnosed health problem, we noted the date of diagnosis and the duration of hospitalization (if hospitalized). We also attributed a code corresponding to the International Classification of Primary Care, 2nd edition (ICPC-2) of the World Organization of National Colleges, Academies and Academic Associations of General Practitioners (WONCA 1998). We then formed four groups of infections: upper respiratory tract infections (URTIs), otitis media, gastro-intestinal (GI) infections, and lower respiratory tract infections (LRTIs). A fifth group labeled *all infections* was formed and included all the infections of the four preceding groups. Because previous studies on OCs and infections in children seem to point towards a greater association between OCs and otitis media compared to other infectious diseases, we decided to exclude ear infections from the URTIs category so that otitis and URTIs can be analyzed independently (Chao et al. 1997, Dewailly et al. 2000, Weisglas-Kuperus et al. 2000). The URTIs category included streptococcal pharyngitis and tonsillitis, acute upper respiratory tract infection not otherwise specified (NOS), acute rhinitis, head cold, nasopharyngitis, pharyngitis, and

coryza. The otitis category included acute suppurative otitis media, otitis media NOS, acute tympanitis, otitis media with effusion, serous otitis media, and glue ear. The LRTIs category included acute bronchitis and bronchiolites, acute lower respiratory infection NOS, chest infection NOS, laryngotracheobronchitis, tracheobronchitis, bacterial and viral pneumonia, bronchopneumonia, influenzal pneumonia, and pneumonitis. The GI infection category included gastrointestinal infection and dysentery with specified organism, diarrhea or vomiting presumed to be infective, dysentery NOS, and gastric flu.

For every health problem identified, we always trusted the diagnosis of the attending physician. When two physicians disagreed, we only recorded the last diagnosis made. In some Inuit communities, nurses are trained to identify and treat benign infections, especially otitis media and upper respiratory tract infections. When the child was not seen by a physician, we recorded the diagnosis of the nurse. We considered two episodes of the same infection type to be separate when there was at least 15 days between the two diagnoses and when it was not specified in the chart that the second episodes was related to the first. When an episode of URTI led to a LRTI, we only included the latter in the analysis. We did not attempt to investigate infectious episodes for which treatment at the health center was not sought by the parents. Data on complications or abnormal events during pregnancy, infant gender and birth weight were also gathered from the medical charts.

Statistical analyses

We assigned a value of half the detection limit of the analytical method when a compound was not detected in a sample. OC concentrations had lognormal distributions and were log-transformed in all analyses. Therefore, contaminants results are presented as geometric means. The correlation between contaminant concentrations was evaluated using Pearson's method on log-transformed values. To evaluate associations between OC exposure and infection incidence rates, we used Poisson regression with quartiles of OC concentration as the main independent variable, and individual incidence rates as the dependent variable (both for bivariate and multivariate analyses). We categorized the exposure using quartiles boundaries, with the first quartile as the group of reference (see table 5.1 for quartiles boundaries). Regression results are, therefore, an estimate of the incidence rate ratios (RRs)

for infants in the three highest quartiles of exposure, when infants in each of these quartiles are compared to infants in first quartile. To test the hypothesis of a dose-response association between incidence rates and OC concentrations (*p*-value for trend), we included the contaminant concentration (log-transformed) directly in the model and treated it as a continuous variable.

The selection of potential confounding variables was based on clinical knowledge and a literature review. Every identified potential confounding variable was tested in the model, but only those influencing the incidence rate ratios by more than 5% were included in the final model. The variables initially excluded from the model were retested one by one in the final model to ensure that their exclusion did not influence the results. The variables included in the final model were: maternal age at delivery (continuous), season of birth, year of birth (category), breastfeeding duration (categories), sex of the infant, socioeconomic status of the caregiver (continuous), smoking during pregnancy (yes/no), number of cigarettes smoked per day during pregnancy (continuous), number of children under 6 years of age living with the infant (continuous), and village of residence. The following variables were excluded from the final model because they did not significantly affect the association of interest: daycare frequentation (ever/never), mean hours per week in a daycare (continuous), maternal omega-3 fatty-acid concentration in blood (continuous), proportion of omega-3 highly unsaturated fatty acids (continuous), number of smokers in the house where the infant resided (continuous), birth weight, gestational age, and reviewer of the medical chart. When postnatal exposure was investigated, the age of the infant when the blood sample was drawn was added in the model. Vaccination coverage was considered as a potential confounding factor. Information on vaccination was gathered through the review of the medical chart but was missing for many children. Preliminary analyses showed that vaccination coverage was not related to contaminant burden. We thus excluded it from the final models.

All modeling results are presented for both the crude model (only exposure categories) and the adjusted model (exposure categories and all the confounding variables mentioned above). Statistical analyses and database management were conducted with the SAS system

8.02 (SAS Institute, Cary, NC, USA). By convention, a *p*-value < 0.05 was considered significant.

Results

Recruitment and participation

During the study period, 417 pregnancies were identified in the targeted communities. Of them, we excluded 47 pregnant women (11.3%) who had already been enrolled in the study during a previous pregnancy and three women (0.7%) due to miscarriage. We were unable to contact nine women (2.2%). Three hundred and fifty eight eligible women were thus asked to participate and 110 refused (30.7%). This refusal rate is comparable to other prospective studies with several interviews in low socioeconomic populations. Of the 248 women willing to participate, we were unable to review the medical charts of 43 infants for the following reasons: 10 (4.0%) moved to another village, 14 (5.6%) were adopted in another village, 11 (4.4%) because of miscarriage or perinatal mortality, and eight (3.2%) because the mother withdrew from the study. Finally, we excluded six (2.4%) participants because no biological samples were available for exposure analysis. A total of 199 participants were included in the final analyses.

Population characteristics

Mothers included in the analysis were mostly from Puvirnituq (45.4%) and Inukjuaq (39.3%). The mean age at delivery was 25.2 years, and most of them smoked during pregnancy (91.4% reported smoking at least one cigarette a day, mean = 10.6 cigarettes/day). Only 2.6% of the infants were not exposed to second-hand smoke during their first year of life. The mean parity was 2.1. There were more males than females (57.6%) and the mean birthweight and length were 3454 g and 50.3 cm, respectively. Breastfeeding was very common and only 12.2% were not breastfed (most of them because they were adopted).

Incidence of infections

Incidence proportions and rates for selected infections are detailed in table 5.1. Otitis media was the most frequent infection diagnosed with a mean of 2.8 episodes per infant-year, followed by URTIs with 2.4 episodes per infant-year. During the first year of life, almost

all infants had at least one episode of otitis (96.0%), and 17.1% had 5 episodes or more. LRTIs required hospitalization in 31.4% of cases. More than half of the infants (56.8%) were hospitalized at least once during their first year of life.

Table 5.1
Incidence proportion and mean infection incidence rate for all participants (n = 199)

Infection	Mean Incidence (episodes / person • year)	Percentage of episodes requiring hospitalization	Percentage of participants who had at least…		
			1 episode	3 episodes	5 episodes
URTIs	2.4 ± 1.7	1.3%	90.0%	42.7%	12.6%
Otitis media	2.8 ± 1.7	0%	96.0%	52.8%	17.1%
GI infections	1.0 ± 1.1	3.4%	58.8%	10.6%	0.5%
LRTIs	1.7 ± 1.7	31.4%	73.4%	26.6%	5.5%

URTI = upper respiratory tract infection; GI = gastro-intestinal; LRTI = lower respiratory tract infection.

Contaminant burden in plasma

Table 5.2 shows the concentration of contaminants in maternal and infant plasma. The geometric mean concentration of the sum of the 14 PCB congeners in maternal plasma was 308 μg/kg ranging from 60 μg/kg to 1951 μg/kg. The concentration of the sum of the PCB congeners was highly correlated with that of PCB congener 153 in maternal plasma ($r = 0.99$). The correlation between cord plasma and maternal plasma was also very high both for the sum the PCB congeners and for PCB congener 153 ($r = 0.95$ and 0.94, respectively). The geometric mean concentration for DDE in maternal plasma was 294 μg/kg ranging from 54 μg/kg to 2269 μg/kg. The correlation between cord and maternal plasma samples for DDE was also very strong ($r = 0.94$). Mean concentrations of PCBs and DDE were lower in infant plasma compared to those in maternal plasma.

Table 5.2
Contaminants concentrations in plasma (lipid-based – µg/kg)

Contaminant	% detected	Geometric mean (95% CI)	Range	Quartile boundaries 1st	2nd	3rd	4th
Maternal plasma (n = 199)							
ΣPCBs	-	308 (279 – 340)	59.6 – 1951	< 190	[190 – 296[	[296 – 500[	≥ 500
PCB 153	100%	102 (91.4 – 113)	14.6 – 709	< 57.6	[57.6 – 98.4[	[98.4 – 170[	≥ 170
DDE	100%	294 (267 – 324)	54.3 – 2269	< 183	[183 – 281[	[281 – 472[	≥ 472
Infant plasma (n = 172)							
ΣPCBs	-	259 (218 – 307)	26.9 – 3801	< 99.0	[99.0 –283[	[283 – 609[	≥ 609
PCB 153	96.5%	76.1 (62.4 – 92.9)	n.d – 1441	< 28.0	[28.0 – 95.3[	[95.3 – 199[	≥ 199
DDE	100%	256 (214 – 307)	15.6 – 4386	< 100	[100 – 355[	[355 – 618[	≥ 618

PCB = polychlorinated biphenyls; DDE = dichlorodiphenyl dichloroethylene; HCB = hexachlorobenzene; n.d. = not detected

Prenatal exposure to PCB 153 and infections

The association between prenatal exposure to PCB 153 and incidence of infections is shown in table 5.3. In preliminary analyses, we found that the associations between OCs and incidence rates were somewhat stronger during the first 6 months of life. Although this study was designed for a 12-month follow-up, we also present the results for the first 6 months of life. Regarding infections during the first 6 months of life and prenatal exposure to PCBs, we observed statistically significant associations only for LRTIs (3rd quartile; RR = 1.54 and 1.68 for the unadjusted and adjusted models, respectively). Although not statistically significant, almost all other RRs were above the unity. When the four types of infections were combined, the relative rates ranged from 1.19 to 1.20 in the unadjusted model, and from 1.17 to 1.27 in the adjusted model. The trend was statistically significant in the adjusted model ($p = 0.04$).

Compared to the first 6 months of life, the effect-size was lower when the first 12 months of life were considered, and only GI infections still pointed towards a positive association. The association was significant for the 3rd quartile in the adjusted model only (RR = 1.59). Globally, rate ratios were similar in the unadjusted and adjusted models.

Table 5.3

Incidence rate ratio of each PCB 153 quartile of prenatal exposure compared to the first quartile

Infection type	Unadjusted model (n = 199)				Adjusted model (n = 177) [a]			
	Incidence rate ratio (95% CI) [b]			*p*-value for trend [c]	Incidence rate ratio (95% CI) [b]			*p*-value for trend [c]
	2nd quartile (*n* = 50)	3rd quartile (*n* = 50)	4th quartile (*n* = 50)		2nd quartile (*n* = 40)	3rd quartile (*n* = 46)	4th quartile (*n* = 45)	
6-month follow-up								
URTIs	1.08 (0.76 - 1.55)	0.98 (0.68 - 1.41)	1.19 (0.84 - 1.68)	0.69	1.08 (0.69 - 1.67)	1.08 (0.71 - 1.65)	1.32 (0.87 - 2.00)	0.22
Otitis media	1.33 (0.85 - 2.07)	1.15 (0.73 - 1.82)	1.30 (0.83 - 2.02)	0.17	1.11 (0.65 - 1.89)	0.99 (0.59 - 1.66)	1.39 (0.82 - 2.35)	0.17
GI infections	1.63 (0.80 - 3.34)	1.31 (0.62 - 2.76)	1.55 (0.75 - 3.20)	0.33	1.89 (0.78 - 4.56)	1.52 (0.65 - 3.54)	1.54 (0.66 - 3.60)	0.38
LRTIs	1.12 (0.71 - 1.76)	1.54 (1.01 - 2.35)*	1.01 (0.63 - 1.61)	0.61	1.16 (0.65 - 2.09)	1.68 (1.00 - 2.81) *	1.18 (0.68 - 2.04)	0.38
All infections [d]	1.19 (0.95 - 1.50)	1.18 (0.94 - 1.48)	1.19 (0.95 - 1.50)	0.14	1.17 (0.88 - 1.55)	1.19 (0.92 - 1.54)	1.27 (0.98 - 1.66)	0.04 *
12-month follow-up								
URTIs	0.93 (0.72 - 1.20)	0.87 (0.67 - 1.13)	1.12 (0.88 - 1.43)	0.81	0.99 (0.71 - 1.36)	0.96 (0.71 - 1.29)	1.23 (0.92 - 1.65)	0.29
Otitis media	1.05 (0.83 - 1.32)	0.97 (0.76 - 1.22)	0.94 (0.75 - 1.20)	0.89	1.02 (0.77 - 1.35)	0.89 (0.68 - 1.17)	0.97 (0.73 - 1.28)	0.89
GI infections	1.27 (0.86 - 1.88)	1.22 (0.82 - 1.82)	1.05 (0.69 - 1.58)	0.81	1.53 (0.94 - 2.49)	1.59 (1.01 - 2.49) *	1.26 (0.78 - 2.04)	0.29
LRTIs	0.88 (0.65 - 1.19)	1.08 (0.81 - 1.45)	0.96 (0.71 - 1.29)	0.48	0.86 (0.57 - 1.28)	1.10 (0.78 - 1.55)	1.03 (0.72 - 1.48)	0.36
All infections [d]	1.00 (0.87 - 1.15)	0.99 (0.86 - 1.14)	1.01 (0.88 - 1.16)	0.67	1.02 (0.86 - 1.21)	1.01 (0.86 - 1.19)	1.08 (0.92 - 1.28)	0.24

PCB = polychlorinated biphenyl; URTI = upper respiratory tract infections; GI = gastro-intestinal; LRTI = lower respiratory tract infections.

[a] Model included mother's age, season of birth, year of birth, breastfeeding duration, sex, socioeconomic status of the caregiver, tobacco use during pregnancy, village of residence and number of children living with the participant.

[b] Incidence rate ratio when the given quartile is compared to the first quartile of exposure (Poisson regression).

[c] *p*-values for trends were calculated by Poisson regression in which the contaminant concentration (lipid-based) was entered as a continuous variable (log-transformed).

[d] We only considered infections with a mean incidence greater than 1.0 episode per year per infant (see methods).

* $p < 0.05$.

Table 5.4

Incidence rate ratio of each DDE quartile of prenatal exposure compared to the first quartile

Infection type	Unadjusted model (n = 199)				Adjusted model (n = 177) [a]			
	Incidence rate ratio (95% CI) [b]			*p*-value for trend [c]	Incidence rate ratio (95% CI) [b]			*p*-value for trend [c]
	2nd quartile (*n* = 50)	3rd quartile (*n* = 50)	4th quartile (*n* = 50)		2nd quartile (*n* = 40)	3rd quartile (*n* = 46)	4th quartile (*n* = 45)	
6-month follow-up								
URTIs	1.50 (1.05 - 2.13)	1.06 (0.72 - 1.55)	1.19 (0.82 - 1.73)	0.91	1.56 (1.05 - 2.33) *	1.15 (0.75 - 1.75)	1.40 (0.90 - 2.16)	0.24
Otitis media	1.27 (0.79 - 2.05)	1.63 (1.04 - 2.57) *	1.50 (0.95 - 2.38)	0.04 *	1.03 (0.59 - 1.77)	1.83 (1.09 - 3.07) *	1.55 (0.90 - 2.68)	0.07
GI infections	2.16 (1.02 - 4.55) *	1.76 (0.81 - 3.82)	1.67 (0.76 - 3.64)	0.34	1.91 (0.84 - 4.35)	1.66 (0.69 - 3.97)	1.35 (0.54 - 3.42)	0.58
LRTIs	1.52 (1.00 - 2.32) *	1.01 (0.64 - 1.59)	1.01 (0.64 - 1.59)	0.75	1.40 (0.86 - 2.29)	1.22 (0.72 - 2.05)	0.96 (0.55 - 1.66)	0.89
All infections [d]	1.49 (1.19 - 1.87) *	1.23 (0.97 - 1.55)	1.25 (0.99 - 1.57)	0.22	1.38 (1.07 - 1.78) *	1.33 (1.03 - 1.73) *	1.27 (0.96 - 1.67)	0.11
12-month follow-up								
URTIs	1.27 (0.98 - 1.63)	1.03 (0.79 - 1.34)	1.11 (0.85 - 1.44)	0.85	1.34 (1.00 - 1.78) *	1.09 (0.81 - 1.47)	1.30 (0.96 - 1.78)	0.27
Otitis media	1.00 (0.79 - 1.27)	1.12 (0.89 - 1.42)	1.08 (0.85 - 1.36)	0.36	0.89 (0.68 - 1.17)	1.08 (0.83 - 1.41)	1.02 (0.76 - 1.35)	0.72
GI infections	1.49 (1.00 - 2.23) *	1.30 (0.86 - 1.96)	1.20 (0.79 - 1.82)	0.98	1.59 (1.03 - 2.47) *	1.27 (0.81 - 2.00)	1.43 (0.87 - 2.34)	0.59
LRTIs	1.15 (0.85 - 1.55)	0.96 (0.70 - 1.30)	1.05 (0.78 - 1.42)	0.89	1.07 (0.75 - 1.51)	0.98 (0.69 - 1.40)	1.00 (0.69 - 1.45)	0.99
All infections [d]	1.17 (1.02 - 1.35) *	1.08 (0.93 - 1.24)	1.09 (0.95 - 1.26)	0.59	1.13 (0.97 - 1.33)	1.08 (0.92 - 1.26)	1.13 (0.95 - 1.34)	0.38

DDE = dichlorodiphenyl trichloroethylene; URTI = upper respiratory tract infections; GI = gastro-intestinal; LRTI = lower respiratory tract infections.

[a] Model included mother's age, season of birth, year of birth, breastfeeding duration, sex, socioeconomic status of the caregiver, tobacco use during pregnancy, village of residence and number of children living with the participant.

[b] Incidence rate ratio when the given quartile is compared to the first quartile of exposure (Poisson regression).

[c] *p*-values for trends were calculated by Poisson regression in which the contaminant concentration (lipid-based) was entered as a continuous variable (log-transformed). [d] We only considered infections with a mean incidence greater than 1.0 episode per year per infant (see methods).

* $p < 0.05$.

Prenatal exposure to DDE and infections

The association between incidence of infections and prenatal exposure to DDE (table 5.4) was similar to that observed for exposure to PCB 153. For the first 6 months of life, we detected significant associations with otitis (RR = 1.63, 3rd quartile) and LRTIs (RR = 1.52, 2nd quartile) in the unadjusted model, and with URTIs (RR = 1.56, 2nd quartile) and otitis (RR = 1.83, 3rd quartile) in the adjusted model. The trend was significant for otitis in the unadjusted model ($p = 0.04$) and borderline significant for in adjusted model ($p = 0.07$). When the four types of infections were combined, we observed significant associations for the 2nd quartile (RR = 1.49) in the unadjusted model, and for the 2nd (RR = 1.38) and 3rd (RR = 1.33) quartiles in the adjusted model. As observed for PCB exposure, almost all RRs were above the unity.

When the first 12 months of life are considered, we observed significant associations for GI infections (RR = 1.49, 2nd quartile) in the unadjusted model, and for URTIs (RR = 1.34, 2nd quartile) and GI infections (RR = 1.59, 2nd quartile) in the adjusted model. For all infections combined, the association reached statistical significance only for the 2nd quartile in the unadjusted model (RR = 1.17).

Postnatal exposure to OCs and infections

We used OCs concentrations in infant plasma to evaluate the effect of postnatal exposure on incidence of infections (sampling done at a median age of 7.0 months). We observed no association between postnatal exposure and the incidence of infections (data not shown). The only significant association was for PCBs (12-month follow-up, 2nd quartile, RR = 1.19) in the unadjusted model, but the statistical significance was lost when adjustment for confounding was done.

Effects of exposure to OCs on hospitalization rate

We found no significant association between pre- or postnatal exposure and incidence rate of hospitalization for LRTIs (data not shown). Statistical power was however poor due to the limited number of admissions.

Discussion

Accidental and occupational exposure to PCBs has already been associated with increased susceptibility to infections in infants. Rogan et al. (1988) observed that mothers who were exposed to PCBs through the consumption of contaminated rice oil (Yu-Cheng accident) reported a higher rate of bronchitis in their children than controls. After examination by two otolaryngologists, the same children were also shown to have a higher prevalence of middle-ear diseases than matched controls (Chao et al. 1997). In Japan, Hara (1985) noted that infants born to women who had handled PCBs in a capacitor factory had a higher incidence of colds and gastrointestinal complaints.

However, evidence of an effect of environmental exposure to OCs on susceptibility to infection in children is scarce and inconsistent. To our knowledge, the first study addressing this question was conducted in the Great Lakes area. The authors observed that fish consumption during pregnancy (a proxy of PCB exposure) was positively associated with colds, earaches, and flu symptoms in infants (Smith 1984). Between 1978 and 1982, Rogan et al. (1987) followed 900 families in North Carolina (USA). They reviewed the medical charts of the children and did not find any evidence of harmful effects of PCBs or DDE during the first year of life. In the Netherlands, Weisglas-Kuperus et al. (1995) observed no association between PCBs and the number of episodes of rhinitis, bronchitis, tonsillitis, and otitis during the first 18 months of life. However, the same group of children was seen at 42 months and current PCB burden was associated with a higher prevalence of recurrent middle-ear infections and of chicken pox (Weisglas-Kuperus et al. 2000). Karmaus et al. (2001) also observed a higher risk of otitis media, but the association was only present with the combined exposure to DDE and PCBs. Finally, our group previously reported that exposed Inuit infants had a higher risk of acute otitis media during the first year of life (3^{rd} tertile of exposure compared to the first). The association was significant with exposure to DDE and HCB but remained above the unity for PCBs, dieldrin and mirex (Dewailly et al. 2000).

In this study, we showed that prenatal exposure to some environmental OC contaminants was possibly associated with a higher incidence rate of infections during the first 6 months

of life. Although the associations were not always statistically significant because of limited statistical power, infants in the highest quartiles of PCBs and DDE exposure had systematically more episodes of infections than their counterparts in the first quartile of exposure. This was mostly observed during the first 6 months of life, as the effect-size was lower when infections during the first 12 months of life were considered. Postnatal exposure to OCs was no longer associated with infection incidence.

In the literature, middle-ear infections are the most consistently reported infections associated with prenatal exposure to OCs. In our study, the strongest dose-response relationship was seen with ear infections. However, it is likely that OCs' insults on the developing immune system would result in the increase of incidence of many different types of acute infections, and not only ear infections. Consistent with that assumption, our results showed a higher incidence rate for the four most frequent infections in infants in the higher exposure groups, and the rate ratios were similar to that observed for otitis. Furthermore, when these four types of infections were combined, the association was more stable and the magnitude of the dose-response relationship was increased, compared to that of the four types of infection taken separately.

We also observed that the effect of prenatal exposure was mostly present during the first few months of life and that this effect seemed to vanish after 6 months of life. Furthermore, we found no effect of postnatal exposure to OCs with infections. It has already been suggested that the immune system is vulnerable to immunotoxic compounds during its development, and that high maternal burden during pregnancy and lactation could lead to permanent defects on the infant's immune system (Badesha et al. 1995, Barnett et al. 1987). Our results support the hypothesis of a stronger effect during early infancy, but we were unable to clearly identify any harmful effect persisting after the age of 6 months. After a few months of life, cumulative environmental influences on the immune system may begin to play a larger role, thus increasing the variability of responses to infections. Furthermore, contributions of the OCs exposure via breast milk, entangled with the beneficial effect of breast-feeding on infections, might have masked the effect. This could explain in part the discrepancies in results of other studies on OCs and infections because the age of children

during disease and exposure assessment varied considerably between studies. Further studies are needed to clarify the time period during which environmental exposure to OCs has a detrimental effect on children health.

In this population, plasma concentrations of many environmentally persistent OCs are strongly correlated (Muckle et al. 2001b). The same authors showed that concentrations in cord plasma, maternal plasma, and breast milk samples are also strongly correlated. With such exposure, it is therefore not possible to attribute the effect observed to one specific OC compound, nor are we able to unravel the specific contribution of PCB 153 exposure from DDE exposure. Furthermore, our data did not allow to determine whether the association between DDE and infections was due to an immune modulation property of DDE, to co-linearity with PCB 153, or both.

We used a review of the medical charts to evaluate disease frequency. There is only one health center in each of the three Inuit communities included in this study. Participants almost always go to that heath center when they seek medical attention, and copies of consultations done elsewhere are routinely requested to complete medical charts. We are, therefore, confident that we have reviewed a majority of the medical consultations sought by the participants. Nevertheless, we did not attempt to verify every diagnosis, nor did we try to inquire about infections for which medical attention was not sought by the parents. Furthermore, we did not find a suitable proxy for the propensity to go to the clinic when symptoms were present (health services are free of charge in Canada). Our results are, therefore, likely to be an underestimation of the true incidence. This underestimation is expected to be present for benign infection, but is unlikely to be significant for LRTIs. It is possible that this underestimation was associated with traditional lifestyle, and thus with OC exposure, but the direction of the bias is unknown. However, if such a bias was present, we could assume that it would have persisted beyond 6 months of age. Since RRs for the 12-month follow-up are close to unity, it seemed to have little effect on our results.

Because of the relatively small number of subjects involved (n = 199), our results must be regarded with caution. Many factors can greatly influence the rate of acute infections. We have assessed several potential confounding factors, but unknown factors might still be

present. Specifically, we cannot rule out the possibility that the infants in the lowest exposure group (first quartile) had better general health due to an unknown cause or simply due to chance. This would have resulted in RRs above the unity for the three highest quartiles of exposure without any dose-response association, which is similar to what we observed. This needs to be kept in mind in the interpretation of our results.

The high rate of infectious episodes in young Inuit children has been observed in Northern Canada, the United States (Alaska), and Greenland (Banerji et al. 2001, Holman et al. 2001, Koch et al. 2002, Proulx 1988, Wainwright 1996). Many cultural, environmental, and economical factors contribute to this situation. Our study population is no exception with a mean of almost nine infection-related medical consultations per infant during the first 12 months of life. In the context of such a high rate of infections, rate ratios of around 1.25, like the ones observed in this study, could have a tremendous impact on the public health of this population. This is the second study identifying a possible association between acute infections and prenatal exposure to OCs in Nunavik. However, the relatively small number of subjects raises the possibility of an association that could be due to chance. To further clarify the potential contribution of persisting contaminants in the high infection rate of these children, we are currently conducting another study in which a third cohort of Inuit children from the same population is followed during the first five years of life. Other studies are also needed to identify which immune mechanisms are involved, as well as to better understand the role of maternal passive immunity in these infants. In the meantime, awareness and precautions regarding the selection of marine food items before and during pregnancies, such as the ones of Environnement Québec (2003), are warranted.

Acknowledgments

We are grateful to the Nunavik population for their participation in this research. We thank the medical and health care professionals from the Inuulitsivik Health Center and the nursing stations in Puvirnituk, Inukjuak, and Kuujjuarapik for their assistance in recruiting this cohort. We acknowledge the support of the Nunavik Nutrition and Health Committee, the Municipal Councils of Puvirnituk, Inukjuaq and Kuujjuarapik, the Pauktuutit Inuit Women's Association, and the Nunalituqait Ikaluqatigiitut Association. We are grateful to

Germain Lebel for his involvement in the management of the exposure data, and to Edna Lachance, Christine Bouffard, Karine Poitras, Lisa Chiodo, Colette Couture, and Brenda Tuttle for their involvement in all phases of the data collection and instrument coding processes. We thank Daria Pereg for her valuable inputs during the preparation of the manuscript. The authors declare they have no competing financial interests.

Support

This study was funded by the National Institute of Environmental Health Sciences/National Institutes of Health (United States, R01-ES07902), by the Department of Indian and Northern Affairs of Canada (Northern Contaminants Program), Health Canada, and Hydro-Québec (Environmental Child Health Initiative). F.D. is supported by the Canadian Institutes of Health Research.

Chapitre 6 – Relation entre l'exposition prénatale aux OC et l'incidence d'infections aiguës chez les enfants d'âge préscolaire au Nunavik

Dallaire F., Dewailly E., Vézina C., Muckle G., Weber J.P., Bruneau S., Ayotte P, 2006. Effect of prenatal exposure to organochlorines on incidence of acute respiratory infections in preschool Inuit children. *Environ Health Perspect*, sous presse.

Résumé

Objectif: Évaluer si une exposition environnementale aux biphenyls polychlorés (BPC) est associée avec l'incidence d'infections aiguës chez les enfants inuits d'âge préscolaire. **Devis d'étude** : Les dossiers médicaux de 343 enfants ont été révisés pour une péroide couvrant les 5 premières années de vie. L'association entre la concentration du congénère de BPC 153 dans le plasma de cordon ombilical et l'incidence d'otites moyennes aiguës (OMA) et d'infections des voies respiratoires supérieures (IVRS) et inférieures (IVRI) a été évaluée. **Résultats**: Les taux d'incidence d'OMA et d'IVRI étaient positivement associés avec l'exposition prénatale aux BPC. Comparativement aux enfants du premier quartile d'exposition (les moins exposés), les enfants du quatrième quartile (les plus exposés) avaient un rapport de taux d'incidence de 1,25 ($p < 0{,}001$) et 1,40 ($p < 0{,}001$) pour les OMA et les IVRI respectivement. Il n'y avait pas d'association entre l'exposition prénatale aux BPC et l'incidence d'IVRS et d'hospitalisations. **Conclusion**: L'exposition prénatale aux PBC pourrait être responsable d'une proportion significative d'infections respiratoires chez les enfants de cette population.

Abstract

Objective: To assess whether environmental prenatal exposure to polychlorinated biphenyls (PCBs) is associated with incidence of acute respiratory infections in preschool inuit children. **Study design**: The medical charts of 343 children were reviewed from 0 to 5 years of age. The association between PCB congener 153 concentration in umbilical cord plasma and the incidence rates of acute otitis media (AOM) and of upper and lower

respiratory tract infections (URTIs and LRTIs) was evaluated. **Results**: The incidence rates of AOM and LRTIs were positively associated with prenatal exposure to PCBs. Compared to children in the first quartile of exposure (least exposed), children in forth quartile (most exposed) had rate ratios of 1.25 ($p < 0.001$) and 1.40 ($p < 0.001$) for AOM and LRTIs, respectively. There was no association between prenatal PCB exposure and incidence rate of URTIs or hospitalization. **Conclusion**: Prenatal exposure to PCBs could be responsible of a significant portion of respiratory infections in children of this population.

Introduction

It is well known that Inuit children from Canada, United States and Greenland suffer from a high incidence of respiratory infections, and many authors have identify higher rates of ear infections and lower respiratory tract infections (LRTIs) in Inuit, compared to Caucasian populations (Banerji et al. 2001, Bluestone 1998, Curns et al. 2002, Davidson et al. 1994, Holman et al. 2001, Karron et al. 1999, Koch et al. 2002, Ling et al. 1969, Lowther et al. 2000, Wainwright 1996). Among the factors suspected to be involved in this phenomenon, perinatal exposure to persistent organic pollutants has been incriminated (Dallaire et al. 2004a, Dewailly et al. 2000). The immunotoxic potential of some organochlorine compounds (OCs), such as 2,3,7,8-tetrachlorodibenzo-*p*-dioxin (TCDD) and polychlorinated biphenyls (PCBs), is well known (Belles-Isles et al. 2002, Chang et al. 1982, Hoffman et al. 1986, Lu & Wu 1985, Neubert et al. 1992, Tryphonas et al. 1991a, Tryphonas et al. 1991b). Although their production and use are now banned in many countries, a significant proportion of what has been emitted in the environment is still present in the biota of almost every region of the world (Braune et al. 1999, Burkow & Kallenborn 2000, Macdonald et al. 2000). The high degree of chlorination of OCs renders them resistant to biodegradation. They accumulate in adipose tissue of living organisms and are biomagnified in the food chain (Evans et al. 1991). The highest plasma concentrations were observed in top predator species (Braune et al. 1999, Muir et al. 1999, Skaare et al. 2000), as well as in humans with seafood-rich diets (Bjerregaard et al. 2001, Dewailly et al. 1993, Humphrey et al. 2000, Sjodin et al. 2000).

The Nunavik region is located in the northernmost part of the province of Quebec, Canada. Around 9 600 Inuit inhabit 14 Inuit communities spread out on the Coast line of the Hudson Bay, the Ungava Bay, and the Hudson Strait. For cultural and economical reasons, carnivorous fish and marine mammals constitute an important part of the diet of the Inuit population of Nunavik. Their exposure to food-chain contaminants, such as OCs, is thus proportionally high. Several studies have identified markedly higher concentrations of OCs in adult blood, umbilical cord blood, and breast milk of Nunavik inhabitants, compared to those of the Southern Québec population (Ayotte et al. 1997, Ayotte et al. 2003, Dewailly et al. 1993, Muckle et al. 2001b, Muckle et al. 1998, Rhainds et al. 1999).

In 2000, we published a first study showing an association between perinatal exposure to OCs and acute otitis media in Nunavik Inuit infants (Dewailly et al. 2000). To confirm this association, we then investigated the relation between maternal OCs concentrations and acute respiratory and gastro-intestinal infections in a cohort of 199 infants of the same population (Dallaire et al. 2004a). We found that OCs in maternal plasma was positively associated with incidence of acute infections during the first 6 months of life, but not afterwards. The number of subjects was however small and the associations were not always statistically significant. In order to clarify the possible link between prenatal exposure to OCs and infections in this population, we tested the association between PCBs concentrations in umbilical cord blood and incidence rate of acute respiratory tract infections in a third cohort of preschool children of Nunavik born between 1993 and 1996.

Materials and methods

Study population

Between 1993 and 1996, we monitored the concentrations of OCs and heavy metals in umbilical cord blood of Nunavik newborns (Dewailly et al. 1998a). Four hundred ninety-one unselected pregnant women from the 14 Inuit communities of Nunavik were enrolled in the study. The women were invited to participate at their arrival at one of the two health centers in Nunavik for delivery (Puvirnituq and Kuujjuaq). Women giving birth elsewhere were not included. A sample of cord blood was taken and an interview was conducted with the mothers few months after delivery. When we initiated the present study, children born

to these mothers were between 4 and 7 years old. They were the targeted participants for the current study.

Medical chart review and infection incidence rate

We attempted to locate and review the medical charts of all the children included in the cord blood monitoring program mentioned above. We made a list of all the medical chart numbers available in our database and worked with the staff of the communities' health centers to locate the charts. We then had the charts copied and sent to our research center for review. Five 2nd- and 3rd-year trained medical students reviewed the charts using a standardized questionnaire. For every diagnosis of infection noted in the charts, we recorded the date of diagnosis, whether antibiotics were prescribed, and whether the child was hospitalized. For each infection, we also attributed a code corresponding to the International Classification of Primary Care, 2nd edition (ICPC2) of the World Organization of National Colleges, Academies and Academic Associations of General Practitioners (WONCA 1998). When the child was hospitalized, we recorded the main reason of hospitalization, whether concurrent illnesses were present, the date of hospitalization, the length of stay, and whether the child was transferred to another hospital. For the present study, we only considered ear and respiratory infections. We formed three categories: upper respiratory tract infections (URTI), lower respiratory tract infections (LRTIs), and acute otitis media (AOM). The URTIs category included streptococcal pharyngitis and tonsillitis, acute upper respiratory tract infection not otherwise specified (NOS), acute rhinitis, head cold, nasopharyngitis, pharyngitis, coryza, sinusitis, tonsillitis, laryngitis, tracheitis, croup, and influenza. In the LRTIs category, we included acute bronchitis and bronchiolites, acute lower respiratory infection NOS, chest infection NOS, laryngotracheobronchitis, tracheobronchitis, bacterial and viral pneumonia, bronchopneumonia, influenzal pneumonia, and pneumonitis. For ear infections, only acute otitis media was included. We excluded otitis media with effusion, chronic otitis media and glue ears.

Data collection on confounding factors

The selection of potential confounding variables was based on clinical knowledge and a literature review. We documented perinatal factors using data from the medical charts

review and the postpartum interview. These factors were maternal age at parturition, smoking during pregnancy, sex of the child, parity, vaccination, reviewer of the medical chart, and gestational age. In order to collect information on other factors, we attempted to contact a subgroup of the participants to plan a 5-year follow-up interview. To be eligible, the child had to be aged ≥ 5 years and < 6 years at the time of the visit to their community of residence. Adopted children had to be excluded because the consent form that allowed us to contact them was signed by the biological mother. Participating mothers were interviewed during five one-month trips across Nunavik between 2000 and 2002. The postnatal factors collected during this 5-year interview were breastfeeding duration, crowding (number of residents ÷ number of rooms, and number of children < 6 years old ÷ number of rooms), daycare attendance, number of smokers in the house, and socioeconomic status of the care givers (Hollingshead index).

Determination of OCs in cord blood

In the original cord blood monitoring program, we determined the concentrations of 14 PCBs congeners (International Union of Pure and Applied Chemistry numbers 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183 and 187), of hexachlorobenzene, and of selected chlorinated pesticides and their metabolites [aldrine, α-chlordane, γ-chlordane, *cis*-nonachlor, *p*,*p*′-dichlorodiphenyl dichloroethylene (DDE), *p*,*p*′-DDT, mirex, oxychlordane, *trans*-nonachlor and β-hexachlorocyclohexane] in plasma samples by high-resolution gas chromatography. These were singled out because they have been widely used and are the most environmentally persistent. Plasma samples (2 mL) were extracted, cleaned on florisil columns, taken to a final volume of 100 µL, and analyzed on an HP-5890 series II gas chromatograph equipped with dual-capillary columns and dual Ni-63 electron-capture detectors (Hewlett-Packard, Palo Alto, CA). We identified peaks by their relative retention times obtained on the two columns. The limit of detection was 0.02 µg/L.

Determination of blood lipids

Because OCs are stored mainly in body fat, all contaminants results are expressed on a lipid basis. We measured total cholesterol, free cholesterol and triglycerides in plasma samples by standard enzymatic procedures. Phospholipids concentrations were determined

according to the enzymatic method of Takayama et al. (1977) using a commercial kit (Wako Pure Chemical Industries, Richmond, VA). We estimated the concentrations of total plasma lipids using the formula developed by Phillips et al. (1989).

Estimation of prenatal exposure to OCs

PCB congener 153 in cord blood was used as a proxy measure for the total PCB burden at birth. PCB 153 is the most abundant congener and its concentration is strongly correlated with all the moderate-to-highly chlorinated PCB congeners and with most chlorinated pesticides. For these reasons, it has been shown to be a good marker of exposure to most organochlorines in the Arctic aquatic food-chain (Muckle et al. 2001b). Participants were grouped according to their quartile of PCB 153 concentrations in cord blood. Children in the lowest quartile were used as the group of reference.

Statistical analyses

Contaminants concentrations had lognormal distributions and were log-transformed in all analyses. Therefore, contaminants results are presented as geometric means. We used Poisson regression to evaluate incidence rate ratio using the number of diagnosed episodes of infection during the first 5 years of life as the dependant variable and PCB 153 concentration in cord blood as the main independent variable. For every analyses, we constructed two models: one in which exposure to OCs was treated in categories (quartiles of exposure with the lowest quartile as the group of reference), and one in which it was treated in continuous (log-transformed). The categorical model yielded estimates of the incidence rate ratios (RRs) for infants in the three highest quartiles of exposure, when infants in each quartile are compared to those in the lowest quartile. The continuous model yielded a single RR corresponding to the relative increase in rate for each log increase of the concentration of PCB 153. To assess the influence of OCs on the hospitalization rates for LRTIs, we used the number of hospitalizations before the age of five years as the main dependent variable.

Adjustment for confounding factors was done using multiple regression (Poisson regression). Because data gathered from the 5-year interview was available for a small number of subjects ($n = 94$), we first considered only variables collected from the medical

charts (model A). These variables were tested in the model one by one, but only those influencing the incidence rate ratios by more than 5% were included in the final model. The variables initially excluded were retested one by one in the final model to ensure that their exclusion did not influence the results. For outpatient visits, the variables included in the final model A were maternal age and parity. We then constructed a second model using the same technique, this time using all available variables (model B). The variables included in final model B for outpatient visits were maternal age, parity, breastfeeding duration, crowding, number of smokers in the house, and socioeconomic status of the care giver. Different models were constructed for hospitalization incidence. In final model A for hospitalization, the variables included were sex, in-utero exposure to cigarette, prematurity (term vs. preterm), maternal age, and parity. Model B for hospitalization was abandoned because the number of episodes was small and the model was unstable (several categories with no episodes). Vaccination coverage was considered as a potential confounding factor but the information on vaccination gathered through the review of the medical chart was inconsistent. Because preliminary analyses showed that vaccination coverage was not related to contaminant burden, and because we found no scientific report linking vaccination coverage with OCs exposure, we excluded it from the final analyses.

We used SPSS Data Entry Builder 2.0 for data entry (Chicago, Illinois, United States) and SAS 8.02 (Cary, NC, United States) for database management and statistical analyses. A *p*-value < 0.05 was considered significant.

Results

Participants

Four hundred ninety-one women were included in the initial cord blood monitoring program. Fifty children were initially excluded because contaminants concentrations were not available or because there was not enough information in our database to trace the charts. Of the 441 remaining participants, it was impossible to get the chart of 43 (9.8%) children for various logistical reasons. Among the 398 available charts, 28 (7.0%) were incomplete, 17 (4.3%) families moved out of Nunavik during follow-up, 7 (1.8%) children

died and 3 (0.8%) children were excluded because they suffered from a serious chronic disease. The final analyses included the 343 remaining children.

To be eligible for the 5-year follow-up interview, children had to be aged 5 years and under the care of their biological mother. We excluded 47 children because they were out of the age limit when our team traveled to their village, and 84 children because they were adopted. Out of the 212 eligible children for the 5-year interview, we were unable to contact 68 mothers, and 7 children/mothers were out of town at the time of the visit. We thus contacted 137 mothers. Only one mother refused to participate to the interview, but 10 did not show up to the appointment. Finally, it was impossible to interview 32 mothers, mainly because of travel problems between the communities. We were thus able to interview 94 mothers. Table 6.1 shows the characteristics for all participants, and for those included in the 5-year interview subgroup.

Table 6.1
Characteristics of participants

Characteristics	Mean value (STD) or percentage	
	All infants (n = 343)	5-year interview subgroup (n = 94)
Children		
Sex (male)	49.0%	45.7%
Given to adoption	24.6%	0% [a]
Year of birth		
1994	37.3%	26.6%
1995	32.1%	42.6%
1996	30.6%	30.8%
Hospital of delivery		
Puvirnituq	48.7%	54.3%
Kuujjuaq	51.3%	45.7%
Gestational age		
Mean gestational age	39.1 weeks	39.1 weeks
Preterm (< 37 weeks)	5.3%	4.3%
Village of residence		
Kuujjuaraapik	4.1%	0%
Umiujak	1.2%	2.1%
Inukjuaq	11.8%	24.5%
Puvirnituq	15.6%	18.1%
Akulivik	5.6%	4.3%
Ivujivik	2.9%	4.3%
Salluit	7.7%	1.1%
Kangiqsujuaq	5.9%	3.2%
Quaqtaq	6.7%	5.3%
Kangirsuk	5.6%	2.1%
Tasiujaq	0%	0%
Kuujjuaq	21.5%	24.5%
Kangiqsualujjuaq	10.6%	10.6%
Birthweight	3494 g	3526 g
Length	51.5 cm	51.1 cm
Mothers		
Age	23.7 years	24.5 years
Parity	2.1	2.2

[a] Adoption was a exclusion criterion during the selection of the 5-year interview subgroup.

Contaminants concentrations

Detailed contaminants concentrations in cord blood for these children have been published elsewhere (Dewailly et al. 1998a). On a lipid basis, the geometric mean concentration of the sum of the 14 PCB congeners (ΣPCBs) in cord blood was 323.5 µg/kg. The PCB congener 153, the most abundant, had a mean concentration of 93.6 µg/kg. Based on the

quartiles limits of PCB 153 in cord blood, the mean concentrations for the four quartiles of OCs prenatal exposure were 147.8 µg/kg, 261.8 µg/kg, 395.4 µg/kg, and 708.9 µg/kg for ΣPCBs, and 38.5 µg/kg, 77.7 µg/kg, 120.8 µg/kg, and 229.2 µg/kg for PCB 153.

Infection incidence rates

The medical chart review of the 343 participants allowed us to identify 5354 outpatient visits that led to a diagnosis of respiratory infections before the age of five years. Annualized incidence rates of AOM, URTIs, and LRTIs are shown in table 6.2. In children aged < 2 years, acute otitis media was the most frequently diagnosed infection, followed by URTIs and LRTIs. In children aged 2 years or more, URTIs were more frequent than AOM. Hospitalizations were frequent as 17.4% of outpatient visits for LRTIs led to an admission. The rate of hospitalizations for LRTIs was 303.2, 145.8, and 36.0 hospitalizations per 1000 child-years for children aged 0-11 months, 12-23 months, and 2-5 years, respectively. A complete portrait of outpatient visits, hospitalization rates and selected risk factors for these children will be published separately.

Table 6.2
Incidence rate of acute otitis media and respiratory infections during the first 5 years of life

Infection	Incidence rate (events per 1000 children-year)		
	< 12 months old	**12 to 23 months old**	**24 months to 5 years old**
All respiratory infections	5434 (5066 – 5803)	4466 (4119 – 4814)	1908 (1732 – 2083)
Acute otitis media	2128 (1932 – 2324)	2087 (1885 – 2290)	697 (614 – 779)
Upper respiratory tract infections	1974 (1799 – 2149)	1487 (1338 – 1636)	888 (786 – 991)
Lower respiratory tract infections	1332 (1171 – 1493)	892 (769 – 1015)	323 (276 – 369)

Prenatal exposure and AOM

Table 6.3 presents the association between exposure to PCB 153 and AOM, URTIs, and LRTIs. In the unadjusted model, prenatal exposure to OCs was associated with AOM incidence rates in a dose-response fashion (RRs = 1.13, 1.18, 1.25 for the second, third and fourth quartiles, respectively). In the unadjusted continuous model, we observed a 6.5 % increase of AOM rates for each log increase of PCB 153 concentration. When adjusted according to model A, we observed a higher effect-size with lower *p*-value, compared to that of the unadjusted model. Statistical significance was lost in model B.

Table 6.3
Incidence rate ratio of otitis media, upper respiratory tract, and lower respiratory tract infections according to prenatal exposure to PCB 153

Prenatal exposure model	Rate ratio (95 % CI)		
	AOM	URTIs	LRTIs
Unadjusted model (*n* = 343)			
Continuous (for each log increase)	1.065 (1.002 – 1.131)*	0.943 (0.887 – 1.002)	1.109 (1.019 – 1.208)*
Categories [a]			
Q1 (less exposed)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Q2	1.13 (1.00 – 1.28)*	0.96 (0.86 – 1.08)	1.37 (1.15 – 1.63)*
Q3	1.18 (1.04 – 1.33)*	0.90 (0.80 – 1.02)	1.21 (1.01 – 1.44)*
Q4 (most exposed)	1.25 (1.10 – 1.41)*	1.00 (0.89 – 1.12)	1.40 (1.18 – 1.67)*
Adjusted model A (*n* = 330)			
Continuous (for each log increase)	1.123 (1.052 – 1.199)*	0.995 (0.931 – 1.063)	1.135 (1.036 – 1.243)*
Categories [a]			
Q1 (less exposed)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Q2	1.15 (1.01 – 1.31)*	0.99 (0.87 – 1.12)	1.39 (1.16 – 1.66)*
Q3	1.26 (1.11 – 1.43)*	0.95 (0.83 – 1.07)	1.25 (1.04 – 1.50)*
Q4 (most exposed)	1.37 (1.20 – 1.55)*	1.09 (0.97 – 1.24)	1.44 (1.20 – 1.72)*
Adjusted model B (*n* = 90)			
Continuous (for each log increase)	1.046 (0.902 – 1.213)	0.953 (0.826 – 1.101)	1.112 (0.919 – 1.346)
Categories [a]			
Q1 (less exposed)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Q2	1.08 (0.81 – 1.45)	1.25 (0.95 – 1.64)	2.56 (1.70 – 3.85)*
Q3	0.98 (0.73 – 1.32)	0.87 (0.65 – 1.15)	1.47 (0.95 – 2.26)
Q4 (most exposed)	1.26 (0.96 – 1.67)	1.13 (0.86 – 1.47)	1.71 (1.14 – 2.57)*

[a] Quartiles of PCB 153 concentration in cord blood.
* Statistically significant ($p < 0.05$)

Prenatal exposure and URTIs

For URTIs, we did not observe significant associations in neither model (table 6.3). We observed a weak negative association between URTIs and prenatal exposure to OCs, especially for children in the 3rd quartile of exposure. In the unadjusted continuous model, the association was negative, but not statistically significant.

Prenatal exposure and LRTIs

The highest effect-size was seen with LRTIs (table 6.3). Rate ratios ranged between 1.21 and 1.40 in the unadjusted model, and between 1.47 and 2.56 in adjusted model B. All associations were statistically significant in the unadjusted model, and in adjusted model A. In adjusted model B, RRs were significant for the second and fourth quartiles (RR = 2.56

and 1.71, respectively). Although the continuous models were strongly positive, a dose-response pattern was not obvious in the categorical models.

Prenatal exposure and hospitalization for LRTIs

Association between prenatal exposure to OCs and hospitalization rates for LRTI is shown in table 6.4. We found no statistically significant association. Rate ratios were above 1.0 for the second and third quartile, but not for the fourth quartile. We observed a negative association in the continuous model, but it did not reached statistical significance. As stated in the methods section, when analyses with model B were performed, there were not enough episodes in each categories and the model was unstable. Therefore, only the results from model A are shown.

Table 6.4
Incidence rate ratio of hospitalization for lower respiratory tract infections according to prenatal exposure to PCB 153

Prenatal exposure model	Rate ratio (95 % CI)	
	Unadjusted model (*n* = 343)	**Adjusted model A** (*n* = 290)
Continuous (for each log increase)	0.920 (0.752 – 1.127)	0.976 (0.768 – 1.240)
Categories [a]		
Q1 (less exposed)	1.00 (referent)	1.00 (referent)
Q2	1.32 (0.88 – 1.97)	1.30 (0.80 – 2.11)
Q3	1.25 (0.83 – 1.87)	1.45 (0.91 – 2.33)
Q4 (most exposed)	0.94 (0.61 – 1.45)	1.00 (0.59 – 1.70)

[a] Quartiles of PCB 153 concentration in cord blood.
* Statistically significant ($p < 0.05$)

Discussion

The aim of this study was to identify an association between prenatal exposure to OCs and rate of acute respiratory infections during the first five years of life. We observed that children in the higher quartiles of exposure had a significantly higher incidence rate of outpatient visits for otitis media and LRTIs, but not for URTIs. This is the third study in which a positive association is observed between OCs and respiratory infections incidence or prevalence in this population. In a cohort of 98 breast-fed infants aged < 1 year recruited in 1989-90, we first observed that infants with higher perinatal exposure to OCs through breastfeeding had a higher prevalence of recurrent otitis media compared to that of infants in the lowest exposure group (Dewailly et al. 2000). In a second cohort of 199 infants aged

< 1 year, we found that the incidence rates of ear infections and LRTIs were positively associated with PCB 153 and DDE concentration in maternal blood (Dallaire et al. 2004a). In the later study, the association was present only during the first 6 months of life. The present study confirms the associations previously observed. Furthermore, it shows that the relation between OCs and respiratory infection seems to persist passed the first months of life.

In the scientific literature, higher rates of respiratory and ear infections have been reported in children born to mother accidentally or occupationally exposed to PCBs, compared to controls (Chao et al. 1997, Hara 1985, Rogan et al. 1988). For environmental exposure, the evidences of an harmful effect of OCs on infections incidence in children is not yet clear, as both an association (Karmaus et al. 2001, Smith 1984, Weisglas-Kuperus et al. 2000, Weisglas-Kuperus et al. 2004) and an absence of association (Rogan et al. 1987, Weisglas-Kuperus et al. 1995) have been reported.

Infection incidence rate in children can be affected by several factors, which make the control for confounding difficult. In this study, information of important potential confounding factors were available for only a subgroup of subjects. Because of the small number of participants involved in this subgroup, the regression models were less stable and the RRs were a little more erratic between the exposure groups. However, in general, we observed that adjustment for confounding slightly increased the magnitude of the associations, both in model A and B. This was also true in our previous study, in which several potential confounding factors were documented for most subjects (Dallaire et al. 2004a). We thus can conclude that in this population, the potential factors that have been considered so far did indeed slightly confound the association by pulling the RRs towards 1.0. Therefore, unadjusted associations between OCs and infection rates for this population were likely to be slightly underestimated.

In this study, we used a review of the medical charts to evaluate incidence rates. There is only one health center in each community included in this study. Participants almost always visit that health center when they seek medical attention, and copies of consultations done elsewhere are routinely requested to complete medical charts. We are therefore confident

that we have reviewed a majority of outpatient visits sought by the participants. Nevertheless, we did not attempt to verify every diagnosis, nor did we try to inquire about infections for which medical attention was not sought by the parents. It is therefore important to keep in mind that the incidence rates reported here are underestimated. Although the magnitude of this underestimation is difficult to evaluate, we can assume that it will be related to both the severity of the symptoms and the parent's perception of the disease (Saunders et al. 2003). We cannot exclude the possibility that the propensity to seek medical attention when respiratory symptoms are present was associated with traditional lifestyle, which in turn is known to be associated with OCs concentration in maternal blood (Muckle et al. 2001a). Should this happen with our participants, the direction of the bias that would be introduced would be unknown. We find it improbable, however, that Inuit families with traditional lifestyle would increase their frequency of medical contacts in such a way that the full extend of the observed association would be solely due to this bias, if any.

The environmental exposure to OCs for most populations, including that of Inuit from Nunavik, consists of a complex mixture of persistent lipophilic chlorinated substances. Because plasma concentrations for most of them are closely correlated with each other (Muckle et al. 2001b), it is impossible to determine which of these compounds – or combination of them – is responsible for the association. In our previous study, DDE concentration in maternal plasma was found to be more closely associated with infection incidence rates, compared to PCB 153 concentration (Dallaire et al. 2004a). In the present study, results for DDE exposure are not shown, but were in general similar to that of PCB 153. Although our analyses were conducted using the PCB congener 153 as a proxy of OC exposure, the potential harmful effect on the immune system could be due to other compounds highly correlated to PCB 153 concentration.

Our initial hypothesis stated that exposure to OCs could influence the severity of respiratory infections, thus leading to an increase of hospitalization rates for LRTIs in groups of higher exposure. Similar to what was previously observed in the same population (Dallaire et al. 2004a), we did not find an association between OCs and hospitalization rate

for LRTIs. The statistical power was limited, but the RRs close to 1.0 observed in the continuous models seem to indicate that no clear link was present. If this interpretation is right, it would mean that the increase incidence rate for LRTIs outpatient visits did not translate into an increase number of admissions, meaning that the episodes of LRTIs that could be explained by OCs exposure were fortunately not severe. Had it been the case, we would have observed equivalent RRs for LRTIs outpatient and inpatient events. Further studies are needed to clarify this important issue.

Inuit children from Nunavik are burden by a high rate of respiratory infectious diseases. In a related study on infection incidence conducted with the same cohort, we shown that LRTIs are far more frequent in Nunavik compared to other Canadian populations, and that hospitalizations rate for LRTIs in Nunavik was one of the highest ever reported in recent scientific literature (Dallaire et al. 2004b). If the association between respiratory infection and prenatal exposure to OCs observed in this population is causal, exposure to OCs during development would be responsible for a significant proportion of respiratory infectious episodes in these children. The biologic mechanism of this effect in human environmentally exposed is still obscure. Other studies are needed to identify which immune pathways are affected in exposed children. Risk-benefit assessments concerning the consumption of contaminated food items should also be continued.

Acknowledgment

We are grateful to the Nunavik population for their participation in this research. We are indebted to Germain Lebel for management of the exposure data. We thank Marie-Lise Mercier, Mélanie Gaudreault, Catherine Lalonde, Élisabeth Leblanc, and Valérie Marchand for medical charts review, and Patsy Tulugak and Mary Nulukie for help with charts retrieval and copying. This study was made possible by grants from Indian and Northern Affairs Canada (Northern Contaminants Program). F. Dallaire is supported by the Canadian Institutes of Health Research.

Chapitre 7 – Conclusion générale

Les objectifs principaux de cette thèse étaient d'identifier des approches biologiques et cliniques pour l'évaluation d'effets immunotoxiques chez une population humaine, d'identifier une tendance temporelle de la concentration d'organochlorés et de métaux lourds dans le sang de cordon ombilical d'enfants du Nunavik, de dresser un portrait de l'incidence d'infections aiguës chez les enfants du Nunavik et, finalement, d'évaluer l'association entre l'exposition périnatale aux organochlorés et l'incidence de consultations et d'hospitalisations pour des infections aiguës chez les enfants du Nunavik.

Les méthodes d'évaluation en immunotoxicologie humaine

Le système immunitaire humain est complexe et la majorité de ses composantes se caractérisent par une grande variabilité intra et inter individuelle. En particulier, le système immunitaire, fonctionnant à bas bruit en situation normale, est capable de monter une réponse parfois fulgurante lorsque cela devient nécessaire. Ainsi, l'évaluation d'une composante semble plus facile (et plus valable) lorsque celle-ci est stimulée. Par exemple, un décompte des cellules NK circulantes ne sera pas aussi sensible qu'une étude de l'activité NK lorsque les cellules sont stimulées par la présence d'autres cellules qui sont reconnues comme du non-soi (Laso et al. 1997, Trinchieri 1989).

La revue de littérature du chapitre 2 nous a montré que les meilleures mesures biologiques d'immunotoxicité sont celles qui font intervenir plusieurs mécanismes immunitaires, telles que la réponse vaccinale et les tests d'hypersensibilité retardée. La production d'anticorps spécifiques et la réponse cellulaire d'hypersensibilité retardée, suite au contact avec un haptène étranger, impliquent l'intégrité de nombreux mécanismes, dont certains sont peu connus. L'évaluation de ces deux paramètres est plus efficace et productive qu'une mesure de chacun des mécanismes qu'ils impliquent. En effet, dans le cas où une substance donnée affecterait un de ces mécanismes, il serait possible d'en détecter l'effet même si ce mécanisme est méconnu ou difficile à mesurer. Ces techniques sont relativement sensibles, mais peu spécifiques. Elles seront particulièrement utiles lors de dépistages ou lorsque l'effet

précis d'une substance est mal connu. Par ailleurs, les mesures de paramètres spécifiques deviennent utiles pour confirmer des hypothèses de mécanismes immunotoxiques plus détaillées.

Une altération des mécanismes immunitaires peut conduire à plusieurs types d'effet d'intérêt en santé publique, telle qu'une augmentation de la susceptibilité aux infections ou de l'incidence de cancer et de maladies auto-immunes. La mesure de la susceptibilité aux infections est l'équivalent clinique des tests biologiques mentionnés plus haut, en ce sens qu'elle représente un marqueur d'effet précoce d'immunotoxicité. D'un point de vue populationnel, même une petite atteinte du système immunitaire se traduira par un effet chez certaines personnes plus susceptibles. De plus, cet effet sera précoce du fait du contact quotidien de chacun des membres d'une population avec une multitude d'agents pathogènes. Le principal défi réside alors en l'identification d'une variation d'incidence de maladies infectieuses, cette dernière étant particulièrement sensible aux biais de confusion et à la grande variabilité des critères diagnostiques.

Selon nous, les perspectives de recherche dans ce domaine résident non pas dans l'élaboration de nouveaux tests sophistiqués, mais bien dans l'intégration des essais disponibles à l'intérieur d'études épidémiologiques bien conçues. Plusieurs batteries de tests existent (voir tableau 2.1), mais n'ont que rarement été utilisées (Tryphonas 2001). De plus, les liens entre les effets cliniques et biologiques sont encore méconnus chez l'humain. Le besoin d'études de cohorte à grande échelle, dans lesquelles plusieurs paramètres biologiques et cliniques seraient à l'étude parallèlement, est à notre avis criant.

Exposition prénatale aux organochlorés et tendances temporelles

La démonstration de l'exposition prénatale aux organochlorés chez les enfants inuits n'est plus à faire (Ando et al. 1985, Dewailly et al. 1993, Dewailly et al. 1996, Dewailly et al. 1989, Muckle et al. 2001b, Muckle et al. 1998, Saxena et al. 1981, Van Oostdam et al. 1999). La longue demi-vie de ces substances chlorées fait qu'elles sont encore présentes dans l'environnement, malgré qu'elles soient bannies par de nombreux de pays industrialisés. Au troisième chapitre de cette thèse, il est montré que la concentration d'organochlorés

dans le sang de cordon ombilical de nouveau-nés inuits a diminué entre 1994 et 2000. La diminution de la contamination environnementale, mais aussi les changements d'habitudes alimentaires, sont vraisemblablement responsables de cette tendance.

Plusieurs études ont déjà établi qu'une tendance descendante des concentrations d'organochlorés était présente chez l'animal et l'humain (Georgii et al. 1994, Harris et al. 1999, He et al. 2001, Hebert et al. 1994, Muir et al. 1999, Muir & Norstrom 2000, Noren 1993, Noren & Meironyte 2000, Roose et al. 1998, Ryckman et al. 1994, Schade & Heinzow 1998, Schmitt et al. 1999, Waliszewski et al. 1998). Au Québec, une étude semblable réalisée avec des nouveau-nés de la Basse-Côte-Nord a montré une tendance similaire (voir annexe 1). Le fait d'observer une diminution des concentrations d'organochlorés chez plusieurs espèces animales ainsi que dans différentes populations humaines nous laisse croire que la baisse des concentrations dans l'environnement est en partie responsable de cette tendance. Par ailleurs, les jeunes mères inuites du Nunavik tendent à délaisser l'alimentation traditionnelle au profit de la nourriture commerciale (ou importée). Ce changement contribue vraisemblablement à la tendance observée dans le sang de cordon ombilical. Selon nos calculs, au rythme actuel, la concentration moyenne de BPC et de DDE dans le sang de cordon ombilical diminue de moitié à toutes les sept ou huit années. Cette valeur de « demi-vie environnementale » est du même ordre de grandeur que celles observées dans d'autres études auprès de populations humaines chez qui les changements d'habitudes alimentaires semblaient moins prononcés (Harris et al. 1999, He et al. 2001, Noren 1993, Noren & Meironyte 2000, Schade & Heinzow 1998, Waliszewski et al. 1998). Cette observation nous laisse croire que la diminution des concentrations d'organochlorés dans l'environnement joue un rôle prépondérant dans la tendance observée chez les nouveau-nés inuits. Des études plus poussées sur les modifications de l'alimentation des Inuit dans le temps permettraient de mieux évaluer à quel point la diminution de l'apport d'aliments traditionnels influence la tendance descendante des concentrations d'organochlorés.

Cette diminution de la concentration des organochlorés dans le sang de cordon ombilical des enfants du Nunavik est encourageante. De plus en plus d'études montrent des effets potentiellement néfastes de ces substances lors d'une exposition prénatale. Une diminution

de l'exposition prénatale à ces substances pourrait alors permettre de diminuer ces effets délétères, et ainsi d'améliorer le développement et la santé de ces enfants. Évidemment, cette diminution ne devrait pas être sortie de son contexte et il serait bien mal avisé de s'en réjouir si celle-ci se fait au détriment d'une alimentation nutritive et équilibrée.

Portrait des infections aiguës chez les enfants du Nunavik

Plusieurs études ont été publiées concernant l'incidence élevée des maladies infectieuses dans les communautés inuites (Banerji 2001, Banerji et al. 2001, Butler et al. 1999, Holman et al. 2001, Koch et al. 2002, Ling et al. 1969, Wainwright 1996). Au Nunavik, les problèmes d'otites moyennes chez les enfants sont reconnus depuis longtemps (Baxter 1999, Baxter et al. 1986, Bluestone 1998, Julien et al. 1987, Thérien 1988). Malgré cela, il n'existe pas d'étude dressant un tableau complet des infections chez ces enfants.

Notre étude a montré qu'au Nunavik, l'otite moyenne aiguë était le problème de santé le plus souvent diagnostiqué chez les enfants de moins de 2 ans. Viennent ensuite les infections respiratoires qui, elles, sont plus fréquentes que les otites chez les enfants plus vieux. Nos résultats, lorsque comparés à ceux d'autres études d'incidence, montrent qu'en général, le taux de consultations menant à un diagnostic d'otite moyenne est plus élevé chez les enfants du Nunavik que ceux d'autres populations caucasiennes nord-américaines, mais semblable à ceux de populations amérindiennes et inuites (Curns et al. 2002, Harris et al. 1998, Wang et al. 1999). Wang et collaborateurs (1999) ont utilisé le registre informatisé de consultations médicales de la Saskatchewan pour estimer l'incidence d'infections dans cette province, une méthode pouvant être considérée équivalente à une revue de dossiers médicaux telle que la nôtre. Les auteurs ont rapporté des taux d'otites moyennes semblables à ceux que nous avons observés au Nunavik. Cette donnée nous a paru étonnante. En effet, étant donné que le problème d'otites est reconnu depuis longtemps au Nunavik, nous nous attendions à trouver une incidence plus élevée chez les enfants inuits que chez ceux de la Saskatchewan. À ce stade, il est cependant impossible de savoir si les taux d'otites moyennes sont effectivement semblables dans les deux populations ou si d'autres facteurs influençant les taux de consultations entrent en jeu.

Nos résultats pour les infections des voies respiratoires inférieures montrent des taux d'incidence plus élevés au Nunavik que dans toutes les autres populations récemment étudiées, exception faite du Groenland (Harris et al. 1998, Koch et al. 2003, Koch et al. 2002, Wang et al. 1999). La différence d'incidence entre les autres études et la nôtre est encore plus grande lorsque l'on considère le taux d'hospitalisation (Kozyrskyj & Hildes-Ripstein 2002, Pageau et al. 2003, Wang et al. 1999). Il en ressort que les infections de voies respiratoires inférieures, de par leur nombre et leur sévérité, semblent être particulièrement problématiques au Nunavik. Nous sommes d'avis que la communauté médicale et les autorités de santé publique devraient se pencher sur la question afin de déterminer quels facteurs entrent en ligne de compte et quelles solutions peuvent être envisagées.

Association entre l'incidence d'infections et l'exposition prénatale aux organochlorés

L'étude de Dewailly et collaborateurs (2000) ainsi que celles présentées aux chapitres 5 et 6 de cette thèse montrent toutes une association positive entre l'exposition aux organochlorés et la susceptibilité aux infections aiguës chez les enfants inuits du Nunavik. La puissance statistique de l'étude de cohorte présentée au chapitre 5 n'a cependant pas été suffisante pour identifier des associations significatives. Pour les six premiers mois de vie, la majorité des rapports de taux étaient supérieurs à 1,0, mais peu étaient statistiquement significatifs. Lorsque les 12 premiers mois de vie étaient considérés, les rapports de taux se rapprochaient de l'unité (voir tableaux 5.3 et 5.4). À la lumière des résultats de cette étude, nous avions initialement conclu qu'une association était probablement présente pendant les 6 premiers mois de vie, mais pas après.

Par rapport à l'étude du chapitre 5, la puissance statistique de l'étude de cohorte présentée au chapitre 6 était d'autant augmentée qu'elle comptait plus de participants et couvrait une période de vie plus longue. Nos analyses préliminaires nous montraient que l'augmentation de l'incidence d'otites et d'infections des voies respiratoires inférieures chez les enfants plus exposés semblait persister tout au long des 5 années de suivi. Nous avons donc choisi de construire nos modèles de régression en utilisant tous les épisodes infectieux des 5 premières années de vie, ce qui nous donnait une plus grande puissance statistique. Les résul-

tats finaux de cette étude ont montré que pour les otites moyennes et les infections des voies respiratoires inférieures, l'association avec l'exposition aux organochlorés était positive et statistiquement significative (voir tableau 6.3). D'autre part, autant les modèles traitant l'exposition en continu que ceux la traitant en catégorie montraient des associations significatives. Pour les otites moyennes, un patron dose-réponse était observable dans le modèle catégoriel. Ce patron n'était cependant pas observable pour les infections des voies respiratoires inférieures, et ce malgré une association significative dans le modèle où l'exposition était traitée en continu. Pour les infections des voies respiratoires inférieures, il nous est impossible à ce stade de savoir si un effet dose-réponse est effectivement présent sans que nous ayons pu le détecter, ou s'il existe un effet de type palier suffisamment important pour qu'un modèle en continu montre quand même une association significative.

Nos résultats viennent appuyer la thèse d'un effet délétère d'une exposition aux organochlorés sur le système immunitaire des enfants. Une association entre une exposition accidentelle ou occupationnelle aux BPC et l'incidence d'infections respiratoires a déjà été identifiée (Chao et al. 1997, Hara 1985, Rogan et al. 1988). D'autres études ont aussi montré une association entre une exposition environnementale aux organochlorés et une augmentation de l'incidence d'infections (Karmaus et al. 2001, Smith 1984, Weisglas-Kuperus et al. 2000, Weisglas-Kuperus et al. 2004). Cette association n'a cependant pas été observée par tous (Rogan et al. 1987, Weisglas-Kuperus et al. 1995).

Plusieurs facteurs influencent l'incidence d'infections respiratoires chez les enfants. Dans la population inuite, il semble que les facteurs de confusion que nous avons considérés dans l'étude du chapitre 5 tendent à sous-estimer l'association entre les organochlorés et les infections lorsqu'ils ne sont pas inclus dans le modèle de régression. En effet, nous avons observé que globalement, l'ajustement pour les biais de confusion de ces facteurs augmentait la force des associations (*effect-size*). Toutefois, tous ces facteurs n'ont pu être évalués lors de l'étude présentée au chapitre 6. Néanmoins, il nous apparaît improbable que l'association observée entre l'exposition aux organochlorés et l'incidence d'infections puisse n'être due qu'à un biais de confusion. Au contraire, ce biais de confusion, s'il est présent, aura probablement sous-estimé l'association mesurée.

Cela étant dit, nous ne pouvons exclure la possibilité que d'autres biais de confusion soient présents dans nos résultats. En particulier, il est possible que la propension à consulter un médecin lors de symptômes d'infections respiratoires soit associée au style de vie traditionnel inuit, lequel est associé à l'exposition prénatale aux organochlorés. Il n'existe cependant pas de données fiables qui puissent nous indiquer la direction ou l'intensité de ce biais. Il nous apparaît par contre improbable que la fréquence de contacts médicaux des familles inuites plus traditionnelles soit à ce point augmentée qu'elle puisse biaiser nos résultats au point d'être responsable d'une partie significative de l'association observée.

Les Inuit, de par leur alimentation, sont exposés à un mélange complexe d'organochlorés. Les concentrations plasmatiques de la majorité des congénères de BPC persistants sont corrélées entre elles, en plus d'être corrélées à celles de plusieurs pesticides chlorés (Muckle et al. 2002). Parmi les BPC, le congénère 153 est le plus abondant. Il a déjà été montré que la concentration plasmatique du BPC 153 était un bon marqueur d'exposition à la plupart des organochlorés dans l'Arctique (Muckle et al. 2002). L'autocorrélation de ces composés est cependant telle qu'il est impossible de distinguer l'effet individuel des divers composés. Ainsi, bien que les modèles statistiques de nos études soient construits en utilisant la concentration de BPC 153, il est plausible que l'effet soit dû à d'autres composés ou, plus probablement, à la somme des effets de plusieurs de ces composés.

À la lumière des résultats de nos études, nous concluons qu'il existe une association entre l'exposition prénatale aux organochlorés et l'incidence d'otites moyennes et d'infections des voies respiratoires inférieures. L'hypothèse biologique étant une dysfonction de plusieurs composantes du système immunitaire, il est probable que les taux d'incidence d'autres types d'infections soient aussi associés à l'exposition aux organochlorés. Considérant que cette association a été observée par d'autres auteurs dans d'autres populations, qu'elle est supportée par des études expérimentales et qu'elle montre un patron dose-réponse, nous croyons qu'elle puisse être causale. Ces études auront ainsi permis d'identifier un des facteurs responsables de l'incidence élevée d'infections aiguës dans cette population.

Perspectives

En acceptant la thèse que l'exposition prénatale aux organochlorés soit responsable d'une partie du taux d'incidence d'infections respiratoires aiguës dans cette population, l'étape suivante serait de réduire cette exposition au minimum. Or, si l'alimentation traditionnelle inuite est associée à une forte exposition aux organochlorés, elle comporte aussi de nombreux avantages. Elle est porteuse de précieux nutriments que l'on retrouve en moindre quantité dans la nourriture importée, en plus d'assurer une continuité et une identité culturelle d'importance inestimable. Les études de risque-bénéfice dans cette situation sont incontournables avant de pouvoir établir des stratégies visant à réduire l'exposition aux organochlorés. Par ailleurs, nous ne pouvons que nous réjouir face à la diminution constante de l'exposition prénatale aux organochlorés.

Les organochlorés ne sont pas les seuls responsables de l'incidence d'infections chez ces enfants. Plusieurs autres facteurs jouent un rôle important, dont la majorité reste encore méconnue. En particulier, les annexes 2 et 3 de cette thèse montrent que les enfants du Nunavik naissent avec des taux de vitamine A circulante relativement faibles et qu'une concentration basse en vitamine A à la naissance est associée à une augmentation de l'incidence d'infections respiratoires. Cette association est indépendante de celle entre les infections et les organochlorés. Il nous semble important de continuer à identifier les facteurs responsables du haut taux d'infections et de cibler des stratégies de prévention efficaces qui pourraient être adaptées à la réalité du nord québécois.

Finalement, bien que le contexte nordique du Nunavik rende les expérimentations de laboratoire plus difficiles et coûteuses, il est essentiel de continuer les efforts visant à mieux comprendre quelles composantes du système immunitaire sont préférentiellement touchées chez ces enfants. Une étude exhaustive des mécanismes immunitaires permettrait également d'identifier chez ces enfants certaines particularités du système immunitaire qui pourraient être en partie responsable de la susceptibilité élevée aux infections.

Chapitre 8 – Bibliographie

Ando M, Hirano S, Itoh Y. 1985. Transfer of hexachlorobenzene (HCB) from mother to newborn baby through placenta and milk. *Arch Toxicol* 56(3):195-200.

Antonaci S, Jirillo E, Bonomo L. 1987. Immunoregulation in aging. *Diagn Clin Immunol* 5(2):55-61.

Aoki Y. 2001. Polychlorinated biphenyls, polychlorinated dibenzo-p-dioxins, and polychlorinated dibenzofurans as endocrine disrupters--what we have learned from Yusho disease. *Environ Res* 86(1):2-11.

Ayotte P, Dewailly É, Ryan JJ, Bruneau S, Lebel G. 1997. PCBs and dioxin-like compounds in plasma of adult Inuit living in Nunavik (Arctic Quebec). *Chemosphere* 34:1459-1468.

Ayotte P, Muckle G, Jacobson JL, Jacobson SW, Dewailly E. 2003. Assessment of pre- and postnatal exposure to polychlorinated biphenyls: lessons from the inuit cohort study. *Environ Health Perspect* 111(9):1253-1258.

Babcock GF, Taylor AF, Hynd BA, Sramkoski RM, Alexander JW. 1987. Flow cytometric analysis of lymphocyte subset phenotypes comparing normal children and adults. *Diagn Clin Immunol* 5(4):175-179.

Baccarelli A, Mocarelli P, Patterson DG, Jr., Bonzini M, Pesatori AC, Caporaso N, et al. 2002. Immunologic effects of dioxin: new results from Seveso and comparison with other studies. *Environ Health Perspect* 110(12):1169-1173.

Badesha JS, Maliji G, Flaks B. 1995. Immunotoxic effects of exposure of rats to xenobiotics via maternal lactation. Part I 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Int J Exp Pathol* 76(6):425-439.

Baker H, Frank O, Thomson AD, Langer A, Munves ED, De Angelis B, et al. 1975. Vitamin profile of 174 mothers and newborns at parturition. *Am J Clin Nutr* 28(1):59-65.

Banerji A. 2001. High rates of hospitalisation for bronchiolitis in Inuit children on Baffin Island. *Int J Circumpolar Health* 60(3):375-379.

Banerji A, Bell A, Mills EL, McDonald J, Subbarao K, Stark G, et al. 2001. Lower respiratory tract infections in Inuit infants on Baffin Island. *Cmaj* 164(13):1847-1850.

Barnett JB, Barfield L, Walls R, Joyner R, Owens R, Soderberg LS. 1987. The effect of in utero exposure to hexachlorobenzene on the developing immune response of BALB/c mice. *Toxicol Lett* 39(2-3):263-274.

Barreto ML, Santos LM, Assis AM, Araujo MP, Farenzena GG, Santos PA, et al. 1994. Effect of vitamin A supplementation on diarrhoea and acute lower-respiratory-tract infections in young children in Brazil. *Lancet* 344(8917):228-231.

Barrie LA, Gregor D, Hargrave B, Lake R, Muir D, Shearer R, et al. 1992. Arctic contaminants: sources, occurrence and pathways. *Sci Total Environ* 122(1-2):1-74.

Basu S, Sengupta B, Paladhi PK. 2003. Single megadose vitamin A supplementation of Indian mothers and morbidity in breastfed young infants. *Postgrad Med J* 79(933):397-402.

Baxter JD. 1999. Otitis media in Inuit children in the Eastern Canadian Arctic--an overview--1968 to date. *Int J Pediatr Otorhinolaryngol* 49 Suppl 1:S165-168.

Baxter JD, Julien G, Tewfik TL, Ilecki HJ, Crago MB. 1986. Observations on the prevalence of ear disease in the Inuit and Cree Indian school population of Kuujjuaraapik. *J Otolaryngol* 15(1):25-30.

Belles-Isles M, Ayotte P, Dewailly E, Weber JP, Roy R. 2002. Cord blood lymphocyte functions in newborns from a remote maritime population exposed to organochlorines and methylmercury. *J Toxicol Environ Health A* 65(2):165-182.

Benes B, Spevackova V, Cejchanova M, Smid J, Svandova E. 2001. Retrospective study of concentration levels of Pb, Cd, Cu and Se in serum of the Czech population in time period 1970-1999. *Cent Eur J Public Health* 9(4):190-195.

Bhaskaram P. 2002. Micronutrient malnutrition, infection, and immunity: an overview. *Nutr Rev* 60(5 Pt 2):S40-45.

Bidwell J, Keen L, Gallagher G, Kimberly R, Huizinga T, McDermott MF, et al. 1999. Cytokine gene polymorphism in human disease: on-line databases. *Genes Immun* 1(1):3-19.

Bilrha H, Roy R, Moreau B, Belles-Isles M, Dewailly E, Ayotte P. 2003. In vitro activation of cord blood mononuclear cells and cytokine production in a remote coastal population exposed to organochlorines and methyl mercury. *Environ Health Perspect* 111(16):1952-1957.

Biswas R, Biswas AB, Manna B, Bhattacharya SK, Dey R, Sarkar S. 1994. Effect of vitamin A supplementation on diarrhoea and acute respiratory tract infection in children. A double blind placebo controlled trial in a Calcutta slum community. *Eur J Epidemiol* 10(1):57-61.

Bjerregaard P. 1991. Health trends in Greenland 1950-1987. *Arctic Med Res* 50(2):79-82.

Bjerregaard P, Dewailly E, Ayotte P, Pars T, Ferron L, Mulvad G. 2001. Exposure of Inuit in Greenland to organochlorines through the marine diet. *J Toxicol Environ Health A* 62(2):69-81.

Bjerregaard P, Hansen JC. 2000. Organochlorines and heavy metals in pregnant women from the Disko Bay area in Greenland. *Sci Total Environ* 245(1-3):195-202.

Blanchet C, Dewailly E, Ayotte P, Bruneau S, Receveur O, Holub BJ. 2000. Contribution of selected traditional and market foods to the diet of Nunavik inuit women. *Can J Diet Prac Res* 61:50-59.

Bloem MW, Wedel M, Egger RJ, Speek AJ, Schrijver J, Saowakontha S, et al. 1990. Mild vitamin A deficiency and risk of respiratory tract diseases and diarrhea in preschool and school children in northeastern Thailand. *Am J Epidemiol* 131(2):332-339.

Bluestone CD. 1998. Epidemiology and pathogenesis of chronic suppurative otitis media: implications for prevention and treatment. *Int J Pediatr Otorhinolaryngol* 42(3):207-223.

Braune B, Muir D, DeMarch B, Gamberg M, Poole K, Currie R, et al. 1999. Spatial and temporal trends of contaminants in Canadian Arctic freshwater and terrestrial ecosystems: a review. *Sci Total Environ* 230(1-3):145-207.

Bright DA, Dushenko WT, Grundy SL, Reimer KJ. 1995. Evidence for short-range transport of polychlorinated byphenyls in the Canadian Arctic using congenere signatures of PCBs in soils. *Sci Total Environ* 160/161:251-263.

Brownell M, Kozyrkyj A, Roos N, Friesen D, Mayer T, Sullivan K. 2002. Health service utilization by Manitoba children. *Can J Public Health* 93 Suppl 2:S57-62.

Bruneau S, Ayukawa H, Proulx JF, Baxter JD, Kost K. 2001. Longitudinal observations (1987-1997) on the prevalence of middle ear disease and associated risk factors among Inuit children of Inukjuak, Nunavik, Quebec, Canada. *Int J Circumpolar Health* 60(4):632-639.

Burkow IC, Kallenborn R. 2000. Sources and transport of persistent pollutants to the Arctic. *Toxicol Lett* 112-113:87-92.

Burton RC, Ferguson P, Gray M, Hall J, Hayes M, Smart YC. 1983. Effects of age, gender, and cigarette smoking on human immunoregulatory T-cell subsets: establishment of normal ranges and comparison with patients with colorectal cancer and multiple sclerosis. *Diagn Immunol* 1(3):216-223.

Butler JC, Parkinson AJ, Funk E, Beller M, Hayes G, Hughes JM. 1999. Emerging infectious diseases in Alaska and the Arctic: a review and a strategy for the 21st century. *Alaska Med* 41(2):35-43.

Cabral JR, Shubik P, Mollner T, Raitano F. 1977. Carcinogenic activity of hexacholorobenzene in hamsters. *Nature* 269(5628):510-511.

Cartier J. 1996. *Enquête sur la consommation des oeufs en Basse-Côte-Nord*. Sept-îles (Québec, Canada): Régie régionale de la santé et des service sociaux de la Côte-Nord, 15 pp.

Catignani GL, Bieri JG. 1983. Simultaneous determination of retinol and alpha-tocopherol in serum or plasma by liquid chromatography. *Clin Chem* 29(4):708-712.

Cernichiari E, Toribara TY, Liang L, Marsh DO, Berlin MW, Myers GJ, et al. 1995. The biological monitoring of mercury in the Seychelles study. *Neurotoxicology* 16(4):613-628.

Chan V, Greenough A, Cheeseman P, Gamsu HR. 1993. Vitamin A status in preterm and term infants at birth. *J Perinat Med* 21(1):59-62.

Chandra RK. 2002. Nutrition and the immune system from birth to old age. *Eur J Clin Nutr* 56 Suppl 3:S73-76.

Chang KJ, Hsieh KH, Lee TP, Tang SY, Tung TC. 1981. Immunologic evaluation of patients with polychlorinated biphenyl poisoning: determination of lymphocyte subpopulations. *Toxicol Appl Pharmacol* 61(1):58-63.

Chang KJ, Hsieh KH, Tang SY, Tung TC, Lee TP. 1982. Immunologic evaluation of patients with polychlorinated biphenyl poisoning: evaluation of delayed-type skin hypersensitive response and its relation to clinical studies. *J Toxicol Environ Health* 9(2):217-223.

Chao WY, Hsu CC, Guo YL. 1997. Middle-ear disease in children exposed prenatally to polychlorinated biphenyls and polychlorinated dibenzofurans. *Arch Environ Health* 52(4):257-262.

Chen YC, Guo YL, Hsu CC, Rogan WJ. 1992. Cognitive development of Yu-Cheng ("oil disease") children prenatally exposed to heat-degraded PCBs. *Jama* 268(22):3213-3218.

Cohen S, Miller GE, Rabin BS. 2001. Psychological stress and antibody response to immunization: a critical review of the human literature. *Psychosom Med* 63(1):7-18.

Colosio C, Corsini E, Barcellini W, Maroni M. 1999. Immune parameters in biological monitoring of pesticide exposure: current knowledge and perspectives. *Toxicol Lett* 108(2-3):285-295.

Corriel RN, Kniker WT, McBryde JL, Lesourd BM. 1985. Cell-mediated immunity in schoolchildren assessed by multitest skin testing. Normal values and proposed scoring system for healthy children. *Am J Dis Child* 139(2):141-146.

Curns AT, Holman RC, Shay DK, Cheek JE, Kaufman SF, Singleton RJ, et al. 2002. Outpatient and hospital visits associated with otitis media among American Indian and Alaska native children younger than 5 years. *Pediatrics* 109(3):E41-41.

Dallaire F, Dewailly E, Laliberte C, Muckle G, Ayotte P. 2002. Temporal trends of organochlorine concentrations in umbilical cord blood of newborns from the lower north shore of the St. Lawrence river (Quebec, Canada). *Environ Health Perspect* 110(8):835-838.

Dallaire F, Dewailly E, Muckle G, Vézina C, Jacobson SW, Jacobson J, et al. 2004a. Acute infections and environmental exposure to organochlorines in Inuit infants from Nunavik. *Environ Health Perspect* 112(14):1359-1365.

Dallaire F, Dewailly E, Shademani R, Laliberte C, Bruneau S, Rhainds M, et al. 2003. Vitamin A concentration in umbilical cord blood of infants from three separate regions of the province of Quebec (Canada). *Can J Public Health* 94(5):386-390.

Dallaire F, Dewailly E, Vezina C, Muckle G, Bruneau S, Ayotte P. 2004b. Portrait of outpatient visits and hospitalizations for acute infections in Nunavik preschool children. *In preparation*.

Daniel V, Huber W, Bauer K, Suesal C, Conradt C, Opelz G. 2002. Associations of dichlorodiphenyltrichloroethane (DDT) 4.4 and dichlorodiphenyldichloroethylene (DDE) 4.4 blood levels with plasma IL-4. *Arch Environ Health* 57(6):541-547.

Daniell WE, Stockbridge HL, Labbe RF, Woods JS, Anderson KE, Bissell DM, et al. 1997. Environmental chemical exposures and disturbances of heme synthesis. *Environ Health Perspect* 105 Suppl 1:37-53.

Darvill T, Lonky E, Reihman J, Stewart P, Pagano J. 2000. Prenatal exposure to PCBs and infant performance on the fagan test of infant intelligence. *Neurotoxicology* 21(6):1029-1038.

Davidson M, Parkinson AJ, Bulkow LR, Fitzgerald MA, Peters HV, Parks DJ. 1994. The epidemiology of invasive pneumococcal disease in Alaska, 1986-1990--ethnic differences and opportunities for prevention. *J Infect Dis* 170(2):368-376.

Department of Fisheries and Oceans. 2002. *Northern Quebec (Nunavik) Beluga (Delphinapterus leucas) - DFO Science Stock Status Report E4-01*. Mont-Joli: Stock Assessment Regional Office - Fisheries and Oceans Canada, 8 pp.

Descotes J, Nicolas B, Pham E, Vial T. 1996. Sentinel screening for human immunotoxicity. *Arch Toxicol Suppl* 18:29-33.

Descotes J, Nicolas B, Vial T. 1995. Assessment of immunotoxic effects in humans. *Clin Chem* 41(12 Pt 2):1870-1873.

Dewailly E, Ayotte P, Brisson J, Dodin S. 1994. Breast cancer and organochlorines. *Lancet* 344(8938):1707-1708.

Dewailly E, Ayotte P, Bruneau S, Gingras S, Belles-Isles M, Roy R. 2000. Susceptibility to infections and immune status in inuit infants exposed to organochlorines. *Environ Health Perspect* 108(3):205-211.

Dewailly E, Ayotte P, Bruneau S, Laliberte C, Muir DC, Norstrom RJ. 1993. Inuit exposure to organochlorines through the aquatic food chain in arctic Quebec. *Environ Health Perspect* 101(7):618-620.

Dewailly E, Ayotte P, Bruneau S, Lebel G, Levallois P, Weber JP. 2001. Exposure of the Inuit population of Nunavik (Arctic Quebec) to lead and mercury. *Arch Environ Health* 56(4):350-357.

Dewailly E, Ayotte P, Laliberte C, Weber JP, Gingras S, Nantel AJ. 1996. Polychlorinated biphenyl (PCB) and dichlorodiphenyl dichloroethylene (DDE) concentrations in the breast milk of women in Quebec. *Am J Public Health* 86(9):1241-1246.

Dewailly E, Bruneau S, Ayotte P, Lebel G, Muckle G, Rhainds M. 1998a. *Évaluation de l'exposition prénatale aux organochlorés et aux métaux lourds chez les nouveau-nés du Nunavik, 1993-1996*. Beauport: Centre de santé publique de Québec, Université Laval, 74 pp.

Dewailly E, Bruneau S, Ayotte P, Lebel G, Muckle G, Rhainds M. 1998b. *Évaluation de l'exposition prénatale aux organochlorés et aux métaux lourds chez les nouveau-nés du Nunavik, 1993-1996 [in French]*. Beauport: Centre de santé publique de Québec, Université Laval, 74 pp.

Dewailly E, Laliberté C, Lebel G, Ayotte P, Weber J-P, Holub B. 1999. *Évaluation de l'exposition prénatale aux organochlorés et aux métaux lourds et des concentrations en oméga-3 des populations de la Moyenne et Basse-Côte-Nord du Saint-Laurent*. Beauport: Unité de recherche en santé publique, Université Laval, 87 pp.

Dewailly E, Laliberte C, Sauve L, Ferron L, Ryan JJ, Gingras S, et al. 1992. Sea-bird egg consumption as a major source of PCB exposure for communities living along the Gulf of St-Lawrence. *Chemosphere* 25:1251-1255.

Dewailly E, Nantel A, Weber JP, Meyer F. 1989. High levels of PCBs in breast milk of Inuit women from arctic Quebec. *Bull Environ Contam Toxicol* 43(5):641-646.

Dich J, Zahm SH, Hanberg A, Adami HO. 1997. Pesticides and cancer. *Cancer Causes Control* 8(3):420-443.

Dietz R, Pacyna J, Thomas DJ, Asmund G, Gordeev VV, Johansen P, et al. 1998. Heavy metals. In: AMAP Assessment Report: Arctic Pollution Issues - Arctic Monitoring and Assessment Program (AMAP) (R. Dietz, J. Pacyna, eds). Oslo, AMAP**:**859.

Ducoffre G, Claeys F, Bruaux P. 1990. Lowering time trend of blood lead levels in Belgium since 1978. *Environ Res* 51(1):25-34.

Dudley L, Hussey G, Huskissen J, Kessow G. 1997. Vitamin A status, other risk factors and acute respiratory infection morbidity in children. *S Afr Med J* 87(1):65-70.

Dufour R. 1988. The otitis media among Inuit children. *Arctic Medical Research* 47(1):659-665.

Duhaime G. 1985. *De l'igloo au H.L.M.* Québec City: Centre d'étude nordique, Université Laval

Durand AM, Sabino H, Jr., Masga R, Sabino M, Olopai F, Abraham I. 1997. Childhood vitamin A status and the risk of otitis media. *Pediatr Infect Dis J* 16(10):952-954.

Duval B, Thérien F. 1982. Natalité, mortalité et morbidité chez les Inuit du Québec arctique. *Rech Amérind Québec* 12:41-50.

Elenkov IJ, Chrousos GP. 2002. Stress hormones, proinflammatory and antiinflammatory cytokines, and autoimmunity. *Ann N Y Acad Sci* 966:290-303.

Environnement Québec 2003. Guide de consommation du poisson de pêche sportive en eau douce [in French]. 2004.

Erkeller-Yuksel FM, Deneys V, Yuksel B, Hannet I, Hulstaert F, Hamilton C, et al. 1992. Age-related changes in human blood lymphocyte subpopulations. *J Pediatr* 120(2 Pt 1):216-222.

Esser C, Welzel M. 1993. Ontogenic development of murine fetal thymocytes is accelerated by 3,3',4,4'-tetrachlorobiphenyl. *Int J Immunopharmacol* 15(8):841-852.

Evans MS, Noguchi GE, Rice CP. 1991. The biomagnification of polychlorinated biphenyls, toxaphene, and DDT compounds in a Lake Michigan offshore food web. *Arch Environ Contam Toxicol* 20(1):87-93.

Fernandes MD, Queiroz ML. 1999. Measurement of the respiratory burst and chemotaxis in polymorphonuclear leukocytes from anti-ChE insecticides-exposed workers. *Immunopharmacol Immunotoxicol* 21(3):621-633.

Frazer IH, Collins EJ, Fox JS, Jones B, Oliphant RC, Mackay IR. 1985. Assessment of delayed-type hypersensitivity in man: a comparison of the "Multitest" and conventional intradermal injection of six antigens. *Clin Immunol Immunopathol* 35(2):182-190.

Fruhwirth M, Ruedl C, Ellemunter H, Bock G, Wolf H. 1998. Flow-cytometric evaluation of oxidative burst in phagocytic cells of children with cystic fibrosis. *Int Arch Allergy Immunol* 117(4):270-275.

Gehrs BC, Smialowicz RJ. 1999. Persistent suppression of delayed-type hypersensitivity in adult F344 rats after perinatal exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Toxicology* 134(1):79-88.

Georgii S, Bachour G, Failing K, Eskens U, Elmadfa I, Brunn H. 1994. Polychlorinated biphenyl congeners in foxes in Germany from 1983 to 1991. *Arch Environ Contam Toxicol* 26(1):1-6.

Ghebremeskel K, Burns L, Burden TJ, Harbige L, Costeloe K, Powell JJ, et al. 1994. Vitamin A and related essential nutrients in cord blood: relationships with anthropometric measurements at birth. *Early Hum Dev* 39(3):177-188.

Gil A. 2002. Polyunsaturated fatty acids and inflammatory diseases. *Biomed Pharmacother* 56(8):388-396.

Gladen BC, Ragan NB, Rogan WJ. 2000. Pubertal growth and development and prenatal and lactational exposure to polychlorinated biphenyls and dichlorodiphenyl dichloroethene. *J Pediatr* 136(4):490-496.

Godel JC, Basu TK, Pabst HF, Hodges RS, Hodges PE, Ng ML. 1996. Perinatal vitamin A (retinol) status of northern Canadian mothers and their infants. *Biol Neonate* 69(3):133-139.

Grimm H, Mayer K, Mayser P, Eigenbrodt E. 2002. Regulatory potential of n-3 fatty acids in immunological and inflammatory processes. *Br J Nutr* 87 Suppl 1:S59-67.

Grutsch JF, Khasawinah A. 1991. Signs and mechanisms of chlordane intoxication. *Biomed Environ Sci* 4(3):317-326.

Hara I. 1985. Health status and PCBs in blood of workers exposed to PCBs and of their children. *Environ Health Perspect* 59:85-90.

Hardell L, Eriksson M. 1999. A case-control study of non-Hodgkin lymphoma and exposure to pesticides. *Cancer* 85(6):1353-1360.

Harris CA, O'Hagan S, Merson GH. 1999. Organochlorine pesticide residues in human milk in the United Kingdom 1997 - 8. *Hum Exp Toxicol* 18(10):602-606.

Harris JR, Markl J. 1999. Keyhole limpet hemocyanin (KLH): a biomedical review. *Micron* 30(6):597-623.

Harris SB, Glazier R, Eng K, McMurray L. 1998. Disease patterns among Canadian aboriginal children. Study in a remote rural setting. *Can Fam Physician* 44:1869-1877.

He JP, Stein AD, Humphrey HE, Paneth N, Courval JM. 2001. Time trends in sport-caught Great Lakes fish consumption and serum polychlorinated biphenyl levels among Michigan Anglers, 1973-1993. *Environ Sci Technol* 35(3):435-440.

Hebert CE, Norstrom RJ, Simon M, Braune BM, Weseloh DV, Macdonald CR. 1994. Temporal trends and sources of PCDDs and PCDFs in the Great Lakes: Herring Gull monitoring 1981-1991. *Environ Sci Technol* 28:1268-1277.

Herbert TB, Cohen S. 1993. Stress and immunity in humans: a meta-analytic review. *Psychosom Med* 55(4):364-379.

Hickie C, Hickie I, Silove D, Wakefield D, Lloyd A. 1995. Delayed-type hypersensitivity skin testing: normal values in the Australian population. *Int J Immunopharmacol* 17(8):629-634.

Hodgins S. (1997). *Health and what affacts it in Nunavik: how is the situation changing?* Kuujjuaq, Nunavik regional board of health and social services.

Hoffman RE, Stehr-Green PA, Webb KB, Evans RG, Knutsen AP, Schramm WF, et al. 1986. Health effects of long-term exposure to 2,3,7,8-tetrachlorodibenzo-p-dioxin. *Jama* 255(15):2031-2038.

Holman RC, Curns AT, Kaufman SF, Cheek JE, Pinner RW, Schonberger LB. 2001. Trends in infectious disease hospitalizations among American Indians and Alaska Natives. *Am J Public Health* 91(3):425-431.

Holsapple MP. 2002. Autoimmunity by pesticides: a critical review of the state of the science. *Toxicol Lett* 127(1-3):101-109.

Holsapple MP, McNerney PJ, Barnes DW, White KL, Jr. 1984. Suppression of humoral antibody production by exposure to 1,2,3,6,7,8-hexachlorodibenzo-p-dioxin. *J Pharmacol Exp Ther* 231(3):518-526.

Holsapple MP, Morris DL, Wood SC, Snyder NK. 1991. 2,3,7,8-tetrachlorodibenzo-p-dioxin-induced changes in immunocompetence: possible mechanisms. *Annu Rev Pharmacol Toxicol* 31:73-100.

Holub BJ, Bakker DJ, Skeaff CM. 1987. Alterations in molecular species of cholesterol esters formed via plasma lecithin-cholesterol acyltransferase in human subjects consuming fish oil. *Atherosclerosis* 66(1-2):11-18.

Homoe P, Christensen RB, Bretlau P. 1999. Acute otitis media and sociomedical risk factors among unselected children in Greenland. *Int J Pediatr Otorhinolaryngol* 49(1):37-52.

Howell WM, Calder PC, Grimble RF. 2002. Gene polymorphisms, inflammatory diseases and cancer. *Proc Nutr Soc* 61(4):447-456.

Huisman M, Koopman-Esseboom C, Fidler V, Hadders-Algra M, van der Paauw CG, Tuinstra LG, et al. 1995. Perinatal exposure to polychlorinated biphenyls and dioxins and its effect on neonatal neurological development. *Early Hum Dev* 41(2):111-127.

Humphrey HE, Gardiner JC, Pandya JR, Sweeney AM, Gasior DM, McCaffrey RJ, et al. 2000. PCB congener profile in the serum of humans consuming great lakes fish. *Environ Health Perspect* 108(2):167-172.

Humphrey JH, West KP, Jr., Sommer A. 1992. Vitamin A deficiency and attributable mortality among under-5-year-olds. *Bull World Health Organ* 70(2):225-232.

IUIS/WHO working group. 1982. Use and abuse laboratory tests in clinical immunology: critical considerations of eight widely-used diagnostic procedures. Report of an IUIS/WHO working group. *Clin Immunol Immunopathol* 24:122-138.

IUIS/WHO working group. 1988. Laboratory investigations in clinical immunology: methods, pitfalls and clinical indications. A second IUIS/WHO report. *Clin Exp Immunol* 74(3):494-503.

Jacobson JL, Fein GG, Jacobson SW, Schwartz PM, Dowler JK. 1984. The transfer of polychlorinated biphenyls (PCBs) and polybrominated biphenyls (PBBs) across the human placenta and into maternal milk. *Am J Public Health* 74(4):378-379.

Jacobson JL, Jacobson SW. 1996. Intellectual impairment in children exposed to polychlorinated biphenyls in utero. *N Engl J Med* 335(11):783-789.

Jacobson JL, Jacobson SW, Padgett RJ, Brumitt GA, Billings RL. 1992. Effects of prenatal PCB exposure on cognitive processing efficiency and sustained attention. *Develop Psychol* 28(2):297-306.

Jacobson SW, Fein GG, Jacobson JL, Schwartz PM, Dowler JK. 1985. The effect of intrauterine PCB exposure on visual recognition memory. *Child Dev* 56(4):853-860.

Janeway C, Travers P. (1996). *Immunobiology : the immune system in health and disease*. San Francisco, Current Biology.

Johansen P, Pars T, Bjerregaard P. 2000. Lead, cadmium, mercury and selenium intake by Greenlanders from local marine food. *Sci Total Environ* 245(1-3):187-194.

Julien G, Baxter JD, Crago M, Ilecki HJ, Therien F. 1987. Chronic otitis media and hearing deficit among native children of Kuujjuaraapik (Northern Quebec): a pilot project. *Can J Public Health* 78(1):57-61.

Julien MR, Gomes A, Varandas L, Rodrigues P, Malveiro F, Aguiar P, et al. 1999. A randomized, double-blind, placebo-controlled clinical trial of vitamin A in Mozambican children hospitalized with nonmeasles acute lower respiratory tract infections. *Trop Med Int Health* 4(12):794-800.

Kalina M, Puxbaum H, Tsakovski S, Simeonov V. 1999. Time trends in the concentrations of lead in wet precipitation from rural and urban sites in Austria. *Chemosphere* 38(11):2509-2515.

Kantakamalakul W, Jaroenpool J, Pattanapanyasat K. 2003. A novel enhanced green fluorescent protein (EGFP)-K562 flow cytometric method for measuring natural killer (NK) cell cytotoxic activity. *J Immunol Methods* 272(1-2):189-197.

Kapil U, Bhavna A. 2002. Adverse effects of poor micronutrient status during childhood and adolescence. *Nutr Rev* 60(5 Pt 2):S84-90.

Karmaus W, Kuehr J, Kruse H. 2001. Infections and atopic disorders in childhood and organochlorine exposure. *Arch Environ Health* 56(6):485-492.

Karron RA, Singleton RJ, Bulkow L, Parkinson A, Kruse D, DeSmet I, et al. 1999. Severe respiratory syncytial virus disease in Alaska native children. RSV Alaska Study Group. *J Infect Dis* 180(1):41-49.

Kartasasmita CB, Rosmayudi O, Deville W, Demedts M. 1995. Plasma retinol level, vitamin A supplementation and acute respiratory infections in children of 1-5 years old in a developing country. Respiratory Diseases Working Group. *Tuber Lung Dis* 76(6):563-569.

Kay RA. 1996. TCR gene polymorphisms and autoimmune disease. *Eur J Immunogenet* 23(2):161-177.

Kerkvliet N. 2002. Recent advances in understanding the mecanisms of TCDD immunotoxicity. *Int Immunopharmacol* 2:277-291.

Kerkvliet NI. 1995. Immunological effects of chlorinated dibenzo-p-dioxins. *Environ Health Perspect* 103 Suppl 9:47-53.

Kimber I, Dearman RJ. 2002. Immune responses: adverse versus non-adverse effects. *Toxicol Pathol* 30(1):54-58.

Kniker WT, Lesourd BM, McBryde JL, Corriel RN. 1985. Cell-mediated immunity assessed by Multitest CMI skin testing in infants and preschool children. *Am J Dis Child* 139(8):840-845.

Koch A, Molbak K, Homoe P, Sorensen P, Hjuler T, Olesen ME, et al. 2003. Risk factors for acute respiratory tract infections in young Greenlandic children. *Am J Epidemiol* 158(4):374-384.

Koch A, Sorensen P, Homoe P, Molbak K, Pedersen FK, Mortensen T, et al. 2002. Population-based study of acute respiratory infections in children, greenland. *Emerg Infect Dis* 8(6):586-593.

Koopman-Esseboom C, Morse DC, Weisglas-Kuperus N, Lutkeschipholt IJ, Van der Paauw CG, Tuinstra LG, et al. 1994. Effects of dioxins and polychlorinated biphenyls on thyroid hormone status of pregnant women and their infants. *Pediatr Res* 36(4):468-473.

Korrick SA, Altshul LM, Tolbert PE, Burse VW, Needham LL, Monson RR. 2000. Measurement of PCBs, DDE, and hexachlorobenzene in cord blood from infants born in towns adjacent to a PCB-contaminated waste site. *J Expo Anal Environ Epidemiol* 10(6 Pt 2):743-754.

Kozyrskyj AL, Hildes-Ripstein GE. 2002. Assessing health status in Manitoba children: acute and chronic conditions. *Can J Public Health* 93 Suppl 2:S44-49.

Kuhnlein HV, Receveur O, Muir DC, Chan HM, Soueida R. 1995. Arctic indigenous women consume greater than acceptable levels of organochlorines. *J Nutr* 125(10):2501-2510.

Kuhnlein HV, Soueida R. 1992. Use and nutrient composition of traditional Baffin Inuit foods. *Journal of Food Composition and Analysis* 5:112-126.

Kuhnlein HV, Soueida R, Receveur O. 1996. Dietary nutrient profiles of Canadian Baffin Island Inuit differ by food source, season, and age. *J Am Diet Assoc* 96(2):155-162.

Laso FJ, Madruga JI, Giron JA, Lopez A, Ciudad J, San Miguel JF, et al. 1997. Decreased natural killer cytotoxic activity in chronic alcoholism is associated with alcohol liver disease but not active ethanol consumption. *Hepatology* 25(5):1096-1100.

Lawn J, Langner N, Brule D, Thompson N, Lawn P, Hill F. 1998. Food consumption patterns of Inuit women. *Int J Circumpolar Health* 57(Suppl 1):198-204.

Levesque B, Duchesne J-F, Gariépy C, Rhainds M, Dumas P, Scheuhammer AM, et al. 2003. Monitoring of umbilical cord blood lead levels and sources assessment among the Inuit. *Occup Environ Med* 60(9):693-695.

Lindblad BS, Patel M, Hamadeh M, Helmy N, Ahmad I, Dawodu A, et al. 1998. Age and sex are important factors in determining normal retinol levels. *J Trop Pediatr* 44(2):96-99.

Ling D, McCoy RH, Levinson ED. 1969. The incidence of middle ear disease and its educational implications among Baffin Island Eskimo children. *Can J Public Health* 60(10):385-390.

Longnecker MP, Gladen BC, Patterson DG, Jr., Rogan WJ. 2000. Polychlorinated biphenyl (PCB) exposure in relation to thyroid hormone levels in neonates. *Epidemiology* 11(3):249-254.

Longnecker MP, Rogan WJ, Lucier G. 1997. The human health effects of DDT (dichlorodiphenyltrichloroethane) and PCBS (polychlorinated biphenyls) and an overview of organochlorines in public health. *Annu Rev Public Health* 18:211-244.

Looker AC, Johnson CL, Woteki CE, Yetley EA, Underwood BA. 1988. Ethnic and racial differences in serum vitamin A levels of children aged 4-11 years. *Am J Clin Nutr* 47(2):247-252.

Lowther SA, Shay DK, Holman RC, Clarke MJ, Kaufman SF, Anderson LJ. 2000. Bronchiolitis-associated hospitalizations among American Indian and Alaska Native children. *Pediatr Infect Dis J* 19(1):11-17.

Lu YC, Wu YC. 1985. Clinical findings and immunological abnormalities in Yu-Cheng patients. *Environ Health Perspect* 59:17-29.

Luebke B. 2002. Pesticides-induced immunotoxicity: are humans at risk? *Human and Ecological Risk Assessment* 8(2):293-303.

Luster MI, Portier C, Pait DG, Rosenthal GJ, Germolec DR, Corsini E, et al. 1993. Risk assessment in immunotoxicology. II. Relationships between immune and host resistance tests. *Fundam Appl Toxicol* 21(1):71-82.

Luster MI, Portier C, Pait DG, White KL, Jr., Gennings C, Munson AE, et al. 1992. Risk assessment in immunotoxicology. I. Sensitivity and predictability of immune tests. *Fundam Appl Toxicol* 18(2):200-210.

MacCrehan WA, Schonberger E. 1987. Determination of retinol, alpha-tocopherol, and beta-carotene in serum by liquid chromatography with absorbance and electrochemical detection. *Clin Chem* 33(9):1585-1592.

Macdonald RW, Barrie LA, Bidleman TF, Diamond ML, Gregor DJ, Semkin RG, et al. 2000. Contaminants in the Canadian Arctic: 5 years of progress in understanding sources, occurrence and pathways. *Sci Total Environ* 254(2-3):93-234.

Maino VC, Suni MA, Ruitenberg JJ. 1995. Rapid flow cytometric method for measuring lymphocyte subset activation. *Cytometry* 20(2):127-133.

Marti A, Marcos A, Martinez JA. 2001. Obesity and immune function relationships. *Obes Rev* 2(2):131-140.

Michielsen CC, van Loveren H, Vos JG. 1999. The role of the immune system in hexachlorobenzene-induced toxicity. *Environ Health Perspect* 107 Suppl 5:783-792.

Ministère des Travaux publics et des Services gouvernementaux du Canada. 2003. *Guide des collectivités indiennes et inuites du Québec*. Ottawa (Ontario): Ministère des Travaux publics et des Services gouvernementaux du Canada, 145 pp.

Moesgaard F, Lykkegaard Nielsen M, Norgaard Larsen P, Christophersen S, Mosbech H. 1987. Cell-mediated immunity assessed by skin testing (Multitest). I. Normal values in healthy Danish adults. *Allergy* 42(8):591-596.

Moffatt M, O'Neil J, Young TK. 1994. Nutritional patterns of Inuit in the Keewatin region of Canada. *Arctic Med Res* 53(Suppl. 2):298-300.

Moss S. 1997. *Baffin region blood monitoring program results*. Yellowknife (NWT): Baffin Regional Health and Social Services Board

Muckle G, Ayotte P, Dewailly E, Jacobson SW, Jacobson JL. 2001a. Determinants of polychlorinated biphenyls and methylmercury exposure in inuit women of childbearing age. *Environ Health Perspect* 109(9):957-963.

Muckle G, Ayotte P, Dewailly E, Jacobson SW, Jacobson JL (2002). Prenatal exposure to PCBs and birth weight in Northern Quebec Inuit (Canada). The second PCB workshop: Recent advances in the environmental toxicology and health effects, Brno, Tcheque Republic.

Muckle G, Ayotte P, Dewailly EE, Jacobson SW, Jacobson JL. 2001b. Prenatal exposure of the northern Quebec Inuit infants to environmental contaminants. *Environ Health Perspect* 109(12):1291-1299.

Muckle G, Dewailly E, Ayotte P. 1998. Prenatal exposure of Canadian children to polychlorinated biphenyls and mercury. *Can J Public Health* 89 Suppl 1:S20-25.

Muir D, Braune B, DeMarch B, Norstrom R, Wagemann R, Lockhart L, et al. 1999. Spatial and temporal trends and effects of contaminants in the Canadian Arctic marine ecosystem: a review. *Sci Total Environ* 230(1-3):83-144.

Muir DC, Born EW, Koczansky K, Stern GA. 2000. Temporal and spatial trends of persistent organochlorines in Greenland walrus (Odobenus rosmarus rosmarus). *Sci Total Environ* 245(1-3):73-86.

Muir DC, Norstrom RJ. 2000. Geographical differences and time trends of persistent organic pollutants in the Arctic. *Toxicol Lett* 112-113:93-101.

Muir DC, Wagemann R, Hargrave BT, Thomas DJ, Peakall DB, Norstrom RJ. 1992. Arctic marine ecosystem contamination. *Sci Total Environ* 122(1-2):75-134.

Murgueytio PU, Evans RG. 1988. Delayed cutaneous hypersensitivity: multitest CMI reliability assessment in groups of volunteers. *Ann Allergy* 61(6):463-465.

Murphy NJ, Schraer CD, Thiele MC, Boyko EJ, Bulkow LR, Doty BJ, et al. 1995. Dietary change and obesity associated with glucose intolerance in Alaska Natives. *J Am Diet Assoc* 95(6):676-682.

Nagayama J, Okamura K, Iida T, Hirakawa H, Matsueda T, Tsuji H, et al. 1998a. Postnatal exposure to chlorinated dioxins and related chemicals on thyroid hormone status in Japanese breast-fed infants. *Chemosphere* 37(9-12):1789-1793.

Nagayama J, Tsuji H, Iida T, Hirakawa H, Matsueda T, Okamura K, et al. 1998b. Postnatal exposure to chlorinated dioxins and related chemicals on lymphocyte subsets in Japanese breast-fed infants. *Chemosphere* 37(9-12):1781-1787.

Neel NR, Alvarez JO. 1990. Chronic fetal malnutrition and vitamin A in cord serum. *Eur J Clin Nutr* 44(3):207-212.

Neubert R, Golor G, Stahlmann R, Helge H, Neubert D. 1992. Polyhalogenated dibenzo-p-dioxins and dibenzofurans and the immune system. 4. Effects of multiple-dose treatment with 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) on peripheral

lymphocyte subpopulations of a non-human primate (*Callithrix jacchus*). *Arch Toxicol* 66(4):250-259.

Newcombe DS 1992. Immunotoxicology: A new chalenge. In: Clinical immunotoxicology (D. S. Newcombe, N. R. Rose, eds). New York, Raven Press**:**1-8.

Nierenberg DW, Lester DC. 1985. Determination of vitamins A and E in serum and plasma using a simplified clarification method and high-performance liquid chromatography. *J Chromatogr* 345(2):275-284.

Noren K. 1993. Contemporary and retrospective investigations of human milk in the trend studies of organochlorine contaminants in Sweden. *Sci Total Environ* 139-140:347-355.

Noren K, Meironyte D. 2000. Certain organochlorine and organobromine contaminants in Swedish human milk in perspective of past 20-30 years. *Chemosphere* 40(9-11):1111-1123.

Odelram H, Bjorksten B, Leander E, Kjellman NI. 1995. Predictors of atopy in newborn babies. *Allergy* 50(7):585-592.

Olsen OR. 1985. Cultural change and health consequences in Inuit. *Arctic Med Res* 40(1A):28-31.

Oostenbrug GS, Mensink RP, Al MD, van Houwelingen AC, Hornstra G. 1998. Maternal and neonatal plasma antioxidant levels in normal pregnancy, and the relationship with fatty acid unsaturation. *Br J Nutr* 80(1):67-73.

Pageau M, Ferland M, Dery S. 2003. *Our children - Health status of children aged 0-5 years in Nunavik*. Kuujjuaq: Direction de santé publique, Régie régionale de la santé et des services sociaux Nunavik, 366 pp.

Pandey A, Chakraborty AK. 1996. Undernutrition, vitamin A deficiency and ARI morbidity in underfives. *Indian J Public Health* 40(1):13-16.

Patandin S, Lanting CI, Mulder PG, Boersma ER, Sauer PJ, Weisglas-Kuperus N. 1999. Effects of environmental exposure to polychlorinated biphenyls and dioxins on cognitive abilities in Dutch children at 42 months of age. *J Pediatr* 134(1):33-41.

Pawelec G, Solana R. 1997. Immunosenescence. *Immunol Today* 18(11):514-516.

Pedersen BK, Bruunsgaard H, Ostrowski K, Krabbe K, Hansen H, Krzywkowski K, et al. 2000. Cytokines in aging and exercise. *Int J Sports Med* 21 Suppl 1:S4-9.

Pelletier G. 1999. *L'hospitalisation pour soins de courtes durées au Québec - Statistiques évolutives 1982-1983 à 1997-1998*. Québec: Direction générale de la planification stratégique et de l'évaluation, Ministère de la santé et des services sociaux du Québec, 204 pp.

Pereg D, Ryan JJ, Ayotte P, Muckle G, Patry B, Dewailly E (2003). Temporal and spatial changes of brominated diphenyl ethers (BDEs) and other POPs in human milk from Nunavik (Arctic) and southern Quebec. Organohalogen Compounds, Boston, MA, USA.

Phillips DL, Pirkle JL, Burse VW, Bernert JT, Jr., Henderson LO, Needham LL. 1989. Chlorinated hydrocarbon levels in human serum: effects of fasting and feeding. *Arch Environ Contam Toxicol* 18(4):495-500.

Pieters R, Ezendam J, Bleumink R, Bol M, Nierkens S. 2002. Predictive testing for autoimmunity. *Toxicol Lett* 127(1-3):83-91.

Pinnock CB, Douglas RM, Badcock NR. 1986. Vitamin A status in children who are prone to respiratory tract infections. *Aust Paediatr J* 22(2):95-99.

Piriou L, Chilmonczyk S, Genetet N, Albina E. 2000. Design of a flow cytometric assay for the determination of natural killer and cytotoxic T-lymphocyte activity in human and in different animal species. *Cytometry* 41(4):289-297.

Pluim HJ, de Vijlder JJ, Olie K, Kok JH, Vulsma T, van Tijn DA, et al. 1993. Effects of pre- and postnatal exposure to chlorinated dioxins and furans on human neonatal thyroid hormone concentrations. *Environ Health Perspect* 101(6):504-508.

Pluim HJ, Koppe JG, Olie K, Vd Slikke JW, Kok JH, Vulsma T, et al. 1992. Effects of dioxins on thyroid function in newborn babies. *Lancet* 339(8804):1303.

Prodan M, Tulissi P, Perticarari S, Presani G, Franzin F, Pussini E, et al. 1995. Flow cytometric assay for the evaluation of phagocytosis and oxidative burst of polymorphonuclear leukocytes and monocytes in myelodysplastic disorders. *Haematologica* 80(3):212-218.

Proulx JF. 1988. Meningitis in Hudson's Bay, Northern Quebec, Canada. *Arctic Medical Research* 47(1):686-687.

Rhainds M, Levallois P, Dewailly E, Ayotte P. 1999. Lead, mercury, and organochlorine compound levels in cord blood in Quebec, Canada. *Arch Environ Health* 54(1):40-47.

Rogan WJ, Gladen BC. 1992. Neurotoxicology of PCBs and related compounds. *Neurotoxicology* 13(1):27-35.

Rogan WJ, Gladen BC, Hung KL, Koong SL, Shih LY, Taylor JS, et al. 1988. Congenital poisoning by polychlorinated biphenyls and their contaminants in Taiwan. *Science* 241(4863):334-336.

Rogan WJ, Gladen BC, McKinney JD, Carreras N, Hardy P, Thullen J, et al. 1987. Polychlorinated biphenyls (PCBs) and dichlorodiphenyl dichloroethene (DDE) in human milk: effects on growth, morbidity, and duration of lactation. *Am J Public Health* 77(10):1294-1297.

Rondo PH, Abbott R, Rodrigues LC, Tomkins AM. 1995. Vitamin A, folate, and iron concentrations in cord and maternal blood of intra-uterine growth retarded and appropriate birth weight babies. *Eur J Clin Nutr* 49(6):391-399.

Roose P, Cooreman K, Vyncke W. 1998. PCBs in cod (Gadus morhua), flounder (Platichthys flesus), blue mussel (Mytilus edulis) and brown shrimp (Crangon crangon) from the Belgian continental shelf: relation to biological parameters and trend analysis. *Chemosphere* 37(9-12):2199-2210.

Rose NR, de Macario EC, Fahey JL, Friedman H, Penn GM. (1992). *Manual of clinical laboratory immunology*. Washington, DC, American Society for Microbiology.

Rose NR, Margolick JB 1992. The immunological assessment of immunotoxic effects in man. In: Clinical immunotoxicology (D. S. Newcombe, N. R. Rose, eds). New York, Raven Press**:**9-25.

Rosenstreich DL. 1993. Evaluation of delayed hypersensitivity: from PPD to poison ivy. *Allergy Proc* 14(6):395-400.

Ross AC 1996. The relationship between immunocompetence and vitamin A status. In: Vitamin A deficiency : health, survival, and vision (A. Sommer, K. P. West). New York, Oxford University Press**:**251-273.

Ross AC, Stephensen CB. 1996. Vitamin A and retinoids in antiviral responses. *Faseb J* 10(9):979-985.

Roth MD, Baldwin GC, Tashkin DP. 2002. Effects of delta-9-tetrahydrocannabinol on human immune function and host defense. *Chem Phys Lipids* 121(1-2):229-239.

Rothenberg SJ, Schnaas L, Perroni E, Hernandez RM, Ortega JF. 2000. Blood lead secular trend in a cohort of children in Mexico City. II. 1990-1995. *Arch Environ Health* 55(4):245-249.

Rothman KJ, Greenland S. (1998). *Modern epidemiology*. Philadelphia, PA, Lippincott-Raven.

Roy SK, Islam A, Molla A, Akramuzzaman SM, Jahan F, Fuchs G. 1997. Impact of a single megadose of vitamin A at delivery on breastmilk of mothers and morbidity of their infants. *Eur J Clin Nutr* 51(5):302-307.

Rubinstein A, Mizrachi Y, Bernstein L, Shliozberg J, Golodner M, Liu GQ, et al. 2000. Progressive specific immune attrition after primary, secondary and tertiary immunizations with bacteriophage phi X174 in asymptomatic HIV- 1 infected patients. *Aids* 14(4):F55-62.

Ryan JJ, Dewailly E, Gilman A, Laliberte C, Ayotte P, Rodrigue J. 1997. Dioxin-like compounds in fishing people from the Lower North Shore of the St. Lawrence River, Quebec, Canada. *Arch Environ Health* 52(4):309-316.

Ryckman DP, Weseloh DV, Bishop CA. 1994. *Contaminants in Herring Gull Eggs from the Great Lakes: 25 Years of Monitoring Levels and Effects*. Burlington (Ontario): Canadian Wildlife Service, Environment Canada, 9 pp.

Safe SH. 1994. Polychlorinated biphenyls (PCBs): environmental impact, biochemical and toxic responses, and implications for risk assessment. *Crit Rev Toxicol* 24(2):87-149.

Safety W-IPOC. (1984). *Chlordane*. Geneva, United Nations Environment programme, the International Labour Organisation, and the World Health Organization.

Salih HR, Husfeld L, Adam D. 2000. Simultaneous cytofluorometric measurement of phagocytosis, burst production and killing of human phagocytes using Candida albicans and Staphylococcus aureus as target organisms. *Clin Microbiol Infect* 6(5):251-258.

Saunders NR, Tennis O, Jacobson S, Gans M, Dick PT. 2003. Parents' responses to symptoms of respiratory tract infection in their children. *Cmaj* 168(1):25-30.

Saxena MC, Siddiqui MK, Bhargava AK, Murti CR, Kutty D. 1981. Placental transfer of pesticides in humans. *Arch Toxicol* 48(2-3):127-134.

Schade G, Heinzow B. 1998. Organochlorine pesticides and polychlorinated biphenyls in human milk of mothers living in northern Germany: current extent of contamination, time trend from 1986 to 1997 and factors that influence the levels of contamination. *Sci Total Environ* 215(1-2):31-39.

Schisterman EF, Whitcomb BW, Louis GM, Louis TA. 2005. Lipid adjustment in the analysis of environmental contaminants and human health risks. *Environ Health Perspect* 113(7):853-857.

Schmitt CJ, Zajicek JL, May TW, Cowman DF. 1999. Organochlorine residues and elemental contaminants in U.S. freshwater fish, 1976-1986: National Contaminant Biomonitoring Program. *Rev Environ Contam Toxicol* 162:43-104.

Schoel B, Welzel M, Kaufmann SH. 1996. Rapid determination of gamma delta T-cell stimulation by microfluorimetry. *Immunol Lett* 53(2-3):135-139.

Schwartz PM, Jacobson SW, Fein G, Jacobson JL, Price HA. 1983. Lake Michigan fish consumption as a source of polychlorinated biphenyls in human cord serum, maternal serum, and milk. *Am J Public Health* 73(3):293-296.

Selgrade MK. 1999. Use of immunotoxicity data in health risk assessments: uncertainties and research to improve the process. *Toxicology* 133(1):59-72.

Semba RD. 1994. Vitamin A, immunity, and infection. *Clin Infect Dis* 19(3):489-499.

Sempertegui F, Estrella B, Camaniero V, Betancourt V, Izurieta R, Ortiz W, et al. 1999. The beneficial effects of weekly low-dose vitamin A supplementation on acute lower respiratory infections and diarrhea in Ecuadorian children. *Pediatrics* 104(1):e1.

Servais G, Walmagh J, Duchateau J. 1991. Simple quantitative haemolytic microassay for determination of complement alternative pathway activation (AP50). *J Immunol Methods* 140(1):93-100.

Shirali GS, Oelberg DG, Mehta KP. 1989. Maternal-neonatal serum vitamin A concentrations. *J Pediatr Gastroenterol Nutr* 9(1):62-66.

Silverman DI, Reis GJ, Sacks FM, Boucher TM, Pasternak RC. 1990. Usefulness of plasma phospholipid N-3 fatty acid levels in predicting dietary fish intake in patients with coronary artery disease. *Am J Cardiol* 66(10):860-862.

Sjodin A, Hagmar L, Klasson-Wehler E, Bjork J, Bergman A. 2000. Influence of the consumption of fatty baltic sea fish on plasma levels of halogenated environmental contaminants in latvian and swedish Men. *Environ Health Perspect* 108(11):1035-1041.

Skaare JU, Bernhoft A, Derocher A, Gabrielsen GW, Goksoyr A, Henriksen E, et al. 2000. Organochlorines in top predators at Svalbard--occurrence, levels and effects. *Toxicol Lett* 112-113:103-109.

Sleijffers A, Garssen J, Van Loveren H. 2002. Ultraviolet radiation, resistance to infectious diseases, and vaccination responses. *Methods* 28(1):111-121.

Smith BJ. 1984. *PCB levels in human fluids: Sheboygan cas study. Technical report WIS-SG-83-240*. Madison, Winsconsin: University of Winsconsin Sea Grant Institute

Sommer A. 1990. Vitamin A status, resistance to infection, and childhood mortality. *Ann N Y Acad Sci* 587:17-23.

Sommer A. 1997. Vitamin A deficiency, child health, and survival. *Nutrition* 13(5):484-485.

Sommer A, Katz J, Tarwotjo I. 1984. Increased risk of respiratory disease and diarrhea in children with preexisting mild vitamin A deficiency. *Am J Clin Nutr* 40(5):1090-1095.

Sottong PR, Rosebrock JA, Britz JA, Kramer TR. 2000. Measurement of T-lymphocyte responses in whole-blood cultures using newly synthesized DNA and ATP. *Clin Diagn Lab Immunol* 7(2):307-311.

Spector BD, Perry GS, 3rd, Kersey JH. 1978. Genetically determined immunodeficiency diseases (GDID) and malignancy: report from the immunodeficiency--cancer registry. *Clin Immunol Immunopathol* 11(1):12-29.

Steuerwald U, Weihe P, Jorgensen PJ, Bjerve K, Brock J, Heinzow B, et al. 2000. Maternal seafood diet, methylmercury exposure, and neonatal neurologic function. *J Pediatr* 136(5):599-605.

Svensson BG, Hallberg T, Nilsson A, Schutz A, Hagmar L. 1994. Parameters of immunological competence in subjects with high consumption of fish contaminated with persistent organochlorine compounds. *Int Arch Occup Environ Health* 65(6):351-358.

Takayama M, Itoh S, Nagasaki T, Tanimizu I. 1977. A new enzymatic method for determination of serum choline-containing phospholipids. *Clin Chim Acta* 79(1):93-98.

Thérien F. 1988. Otitis and hearing loss among northern Quebec Inuit. *Arctic Med Res* 47(Suppl 1):657-658.

Thomas DJ, Tracey B, Marshall H, Norstrom RJ. 1992. Arctic terrestrial ecosystem contamination. *Sci Total Environ* 122(1-2):135-164.

Thomas PT, Hinsdill RD. 1979. The effect of perinatal exposure to tetrachlorodibenzo-p-dioxin on the immune response of young mice. *Drug Chem Toxicol* 2(1-2):77-98.

Tolba AM, Hewedy FM, al-Senaidy AM, al-Othman AA. 1998. Neonates' vitamin A status in relation to birth weight, gestational age, and sex. *J Trop Pediatr* 44(3):174-177.

Trinchieri G. 1989. Biology of natural killer cells. *Adv Immunol* 47:187-376.

Tryphonas H. 2001. Approaches to detecting immunotoxic effects of environmental contaminants in humans. *Environ Health Perspect* 109 Suppl 6:877-884.

Tryphonas H, Luster MI, Schiffman G, Dawson LL, Hodgen M, Germolec D, et al. 1991a. Effect of chronic exposure of PCB (Aroclor 1254) on specific and nonspecific immune parameters in the rhesus (*Macaca mulatta*) monkey. *Fundam Appl Toxicol* 16(4):773-786.

Tryphonas H, Luster MI, White KL, Jr., Naylor PH, Erdos MR, Burleson GR, et al. 1991b. Effects of PCB (Aroclor 1254) on non-specific immune parameters in rhesus (*Macaca mulatta*) monkeys. *Int J Immunopharmacol* 13(6):639-648.

Underwood BA. 1994. The role of vitamin A in child growth, development and survival. *Adv Exp Med Biol* 352:201-208.

United States National Academy of Science. (1992). *Biologic markers in immunotoxicology - report from the Subcommitte on Immunotoxicology (Committe on Biologic Markers)*. Washington, National Academy Press.

Urban L, Bessenyei B, Marka M, Semsei I. 2002. On the role of aging in the etiology of autoimmunity. *Gerontology* 48(3):179-184.

Van Den Heuvel RL, Koppen G, Staessen JA, Hond ED, Verheyen G, Nawrot TS, et al. 2002. Immunologic biomarkers in relation to exposure markers of PCBs and dioxins in Flemish adolescents (Belgium). *Environ Health Perspect* 110(6):595-600.

Van Loveren H, Germolec D, Koren HS, Luster MI, Nolan C, Repetto R, et al. 1999. Report of the Bilthoven Symposium: Advancement of epidemiological studies in assessing the human health effets of immunotoxic agents in the environment and the workplace. *Biomarkers* 4(2):135-157.

Van Loveren H, Van Amsterdam JG, Vandebriel RJ, Kimman TG, Rumke HC, Steerenberg PS, et al. 2001. Vaccine-induced antibody responses as parameters of the influence of endogenous and environmental factors. *Environ Health Perspect* 109(8):757-764.

Van Oostdam J, Gilman A, Dewailly E, Usher P, Wheatley B, Kuhnlein H, et al. 1999. Human health implications of environmental contaminants in Arctic Canada: a review. *Sci Total Environ* 230(1-3):1-82.

Venjatraman JT, Fernandes G. 1997. Exercise, immunity and aging. *Aging (Milano)* 9(1-2):42-56.

Venkatarao T, Ramakrishnan R, Nair NG, Radhakrishnan S, Sundaramoorthy L, Koya PK, et al. 1996. Effect of vitamin A supplementation to mother and infant on morbidity in infancy. *Indian Pediatr* 33(4):279-286.

Vial T, Nicolas B, Descotes J. 1996. Clinical immunotoxicity of pesticides. *J Toxicol Environ Health* 48(3):215-229.

Villamor E, Fawzi WW. 2000. Vitamin A supplementation: implications for morbidity and mortality in children. *J Infect Dis* 182 Suppl 1:S122-133.

Vorderstrasse BA, Steppan LB, Silverstone AE, Kerkvliet NI. 2001. Aryl hydrocarbon receptor-deficient mice generate normal immune responses to model antigens and are resistant to TCDD-induced immune suppression. *Toxicol Appl Pharmacol* 171(3):157-164.

Vos JG, van Loveren H. 1995. Markers for immunotoxic effects in rodents and man. *Toxicol Lett* 82-83:385-394.

Vulsma T. 2000. Impact of exposure to maternal PCBs and dioxins on the neonate's thyroid hormone status. *Epidemiol* 11(3):239-241.

Wagemann R, Innes S, Richard PR. 1996. Overview and regional and temporal differences of heavy metals in Arctic whales and ringed seals in the Canadian Arctic. *Sci Total Environ* 186(1-2):41-66.

Wainwright RB. 1996. The US Arctic Investigations Program: infectious disease prevention and control research in Alaska. *Lancet* 347(9000):517-520.

Waliszewski SM, Aguirre AA, Infanzon RM, Rivera J, Infanzon R. 1998. Time trend of organochlorine pesticide residues in human adipose tissue in Veracruz, Mexico: 1988-1997 survey. *Sci Total Environ* 221(2-3):201-204.

Wang EE, Einarson TR, Kellner JD, Conly JM. 1999. Antibiotic prescribing for Canadian preschool children: evidence of overprescribing for viral respiratory infections. *Clin Infect Dis* 29(1):155-160.

Ward PA 1992. Flow cytometric analysis of the immune and phagocytic cells. In: Clinical immunotoxicology (D. S. Newcombe, N. R. Rose, eds). New York, Raven Press**:**43-47.

Weisglas-Kuperus N, Patandin S, Berbers GA, Sas TC, Mulder PG, Sauer PJ, et al. 2000. Immunologic effects of background exposure to polychlorinated biphenyls and dioxins in Dutch preschool children. *Environ Health Perspect* 108(12):1203-1207.

Weisglas-Kuperus N, Sas TCJ, Koopman-Esseboom C, Van Der Zwan CW, De Ridder MAJ, Beishuizen A, et al. 1995. Immunologic effects of background prenatal and posnatal exposure to dioxins and polychlorinated biphenyls in Dutch infants. *Ped Res* 38(3):404-410.

Weisglas-Kuperus N, Vreugdenhil HJ, Mulder PG. 2004. Immunological effects of environmental exposure to polychlorinated biphenyls and dioxins in Dutch school children. *Toxicol Lett* 149(1-3):281-285.

West KP, Jr., Howard GR, Sommer A. 1989. Vitamin A and infection: public health implications. *Annu Rev Nutr* 9:63-86.

White KL, Jr., Lysy HH, McCay JA, Anderson AC. 1986. Modulation of serum complement levels following exposure to polychlorinated dibenzo-p-dioxins. *Toxicol Appl Pharmacol* 84(2):209-219.

Wietlisbach V, Rickenbach M, Berode M, Guillemin M. 1995. Time trend and determinants of blood lead levels in a Swiss population over a transition period (1984-1993) from leaded to unleaded gasoline use. *Environ Res* 68(2):82-90.

Wilson CB. 1986. Immunologic basis for increased susceptibility of the neonate to infection. *J Pediatr* 108(1):1-12.

WONCA. (1998). *International classification of primary care (ICPC-2)*. New-York, Oxford University Press.

World Health Organization. (1996). *Principles and methods for assessing direct immunotoxicity associated with exposure to chemical*. Geneva, World Health Organisation.

Yeum KJ, Ferland G, Patry J, Russell RM. 1998. Relationship of plasma carotenoids, retinol and tocopherols in mothers and newborn infants. *J Am Coll Nutr* 17(5):442-447.

Yu ML, Hsu CC, Gladen BC, Rogan WJ. 1991. In utero PCB/PCDF exposure: relation of developmental delay to dysmorphology and dose. *Neurotoxicol Teratol* 13(2):195-202.

Annexe 1 – Tendances temporelles des concentrations d'organochlorés dans le sang de cordon ombilical de nouveau-nés de la Basse-Côte-Nord du Saint-Laurent

Dallaire F., Dewailly E., Laliberte C., Muckle G., Ayotte P., 2002. Temporal trends of organochlorine concentrations in umbilical cord blood of newborns from the lower north shore of the St. Lawrence river (Quebec, Canada). *Environ Health Perspect* 110(8):835-838.

Résumé

Cette étude décrit les tendances temporelles des concentrations d'organochlorés [14 congénères de biphényls polychlorés (BPC) et 11 pesticides chlorés] dans le sang de cordon ombilical de nouveau-nés d'une région côtière isolée du Canada. Nous avons dosé les BPC, les chorldanes, le dichlorodiphényltrichloroéthane (DDT), le dichlorodiphényldichloroéthylène (DDE), l'hexachlorobenzène (HCB) et les acides gras oméga-3 (n-3) dans 408 échantillons de sang de cordon ombilical d'enfants nés entre 1993 et 2000. Nous avons aussi recueilli de l'information sur les mères (âge, résidences présente et passées, ethnie, habitudes tabagiques pendant la grossesse et l'allaitement des naissances antérieures). De 1993 à 2000, les concentrations moyennes de BPC, chlordanes, DDT/DDE et HCB dans le sang de cordon ombilical ont diminuées de 63 %, 25 %, 66 % et 69 % respectivement ($p < 0.0001$). L'analyse en régression multiple avec l'année de naissance comme variable indépendante principale a révélé une forte diminution exponentielle statistiquement significative pour tous les contaminants, dans tous les groupes d'âge et pour toutes les ethnies. Aucune tendance mensuelle ou saisonnière n'a été détectée. Nous avons utilisé les acides gras n-3 en tant que substitut de la consommation maternelle de poisson. La consommation de poisson n'a que faiblement diminué entre 1993 et 2000 et cette diminution n'a pas contribué significativement à la réduction d'organochlorés. Ces résultats montrent que l'exposition prénatale aux organochlorés a diminué significativement entre 1993 et 2000 dans cette population.

Abstract

This study describes the time trends of organochlorines [OCs; 14 polychlorinated biphenyls (PCBs) and 11 chlorinated pesticides] in umbilical cord plasma of newborns in a remote Canadian coastal population. We analyzed 408 cord blood samples collected between 1993 and 2000 for PCBs, chlordanes, dichlorodiphenyltrichloroethane (DDT), dichlorodiphenyldichloroethylene (DDE), hexachlorobenzene (HCB), and n-3 fatty acids. We also gathered information on the mothers (age, past and present residence, ethnic group, use of tobacco during pregnancy and breastfeeding during previous pregnancies). From 1993 to 2000, mean concentrations of PCBs, chlordanes, DDT/E and HCB in cord blood decreased by 63 %, 25 %, 66 % and 69 % respectively ($p < 0.0001$). Multiple regression analysis with the year of birth as the main independent variable yielded a strong significant exponential decrease for all contaminants (in all age and ethnic groups). No monthly or seasonal pattern was detected. We used n-3 fatty acids concentration as a surrogate of maternal fish consumption. Fish consumption declined only slightly between 1993 and 2000 but this decrease did not significantly contribute to the reduction of organochlorines. These results show that prenatal exposure to persistent OCs has declined significantly between 1993 and 2000 in this population.

Introduction

Persistent organochlorines (OCs) such as chlorinated pesticides and polychlorinated biphenyls (PCBs) have been emitted by industrialized countries for many years. Substantial regulatory actions have been taken since the late 1970s to limit their emission but they are still released because of improper storage and ongoing use in certain parts of the world. These substances bioaccumulate in fat tissues, are biomagnified in the food chain and reach high levels in top predator species (Braune et al. 1999, Muir et al. 1999, Muir et al. 1992). Human populations with seafood-rich diets have elevated concentrations of OCs in their blood (Bjerregaard et al. 2001, Dewailly et al. 1993, Humphrey et al. 2000, Ryan et al. 1997, Sjodin et al. 2000). Because OCs readily cross the placental barrier (Ando et al. 1985, Jacobson et al. 1984, Saxena et al. 1981), high concentrations in the blood of pregnant women usually cause significant prenatal exposure for the fetus (Moss 1997, Muckle et al. 2001a, Schwartz et al. 1983).

A general downward trend of the concentrations of OCs in tissue of wild animals and human has been observed in the last decade. Levels of dichlorodiphenyl trichloroethane (DDT) and PCBs decreased in tissues of fresh-water fishes between 1976 and 1986 (Schmitt et al. 1999). In Herring Gull eggs from the Great Lakes, levels of PCBs and dichlorodiphenyldichloroethylene (DDE) also declined until the mid 1980s, after peaking in the mid 1970s (Hebert et al. 1994, Ryckman et al. 1994). A similar trend has been observed in the concentrations of PCBs in fat tissues of polar bears, ringed seals, and sea birds in the Canadian arctic (Muir et al. 1999, Muir & Norstrom 2000). In environmentally exposed humans, levels of PCBs in breast milk have dropped in the last 15 years in Germany (Schade & Heinzow 1998) and Sweden (Noren 1993, Noren & Meironyte 2000). In Michigan, PCB blood concentrations increased between 1973 and 1983 but declined between 1983 and 1993 in a control population but not in fish eaters (He et al. 2001). No recent study has been published on time trends of OCs environmental exposure for the fetus.

The Lower North Shore of the Saint-Lawrence River (Québec, Canada) is a remote coastal region of 15 small communities scattered along 400 km of marine coastline and inhabited by both Caucasians and Natives (mainly Montagnais). Although consumption of fish is common in this region, the principal source of exposure to OCs comes from the consumption of sea-bird eggs (Dewailly et al. 1992). Since 1993, we have been collecting cord blood samples to monitor OCs prenatal exposure and temporal trends in this region. In light of the general downward trend of OCs environmental exposure of the last years, we report here the time trends in prenatal exposure to PCBs and chlorinated pesticides for infants born between 1993 and 2000.

Materials and Methods

Subjects and blood sampling

Our target population consisted of pregnant women who have lived for at least 5 years in one of the 15 small communities of the Lower North Shore region of the Saint-Lawrence River (Québec, Canada, see figure 9.1). We invited mothers admitted for full-term delivery in the two regional health centers of the region to participate in the study. We conducted the

first phase of this program between April 1993 and December 1997, and the second phase between November 1999 and January 2001. Of 432 women eligible for the study, 403 (93 %) agreed to participate. We did not try to include women for whom the delivery was at risk or when special or urgent medical care was needed. Therefore, a slight bias towards healthier pregnancy may be present.

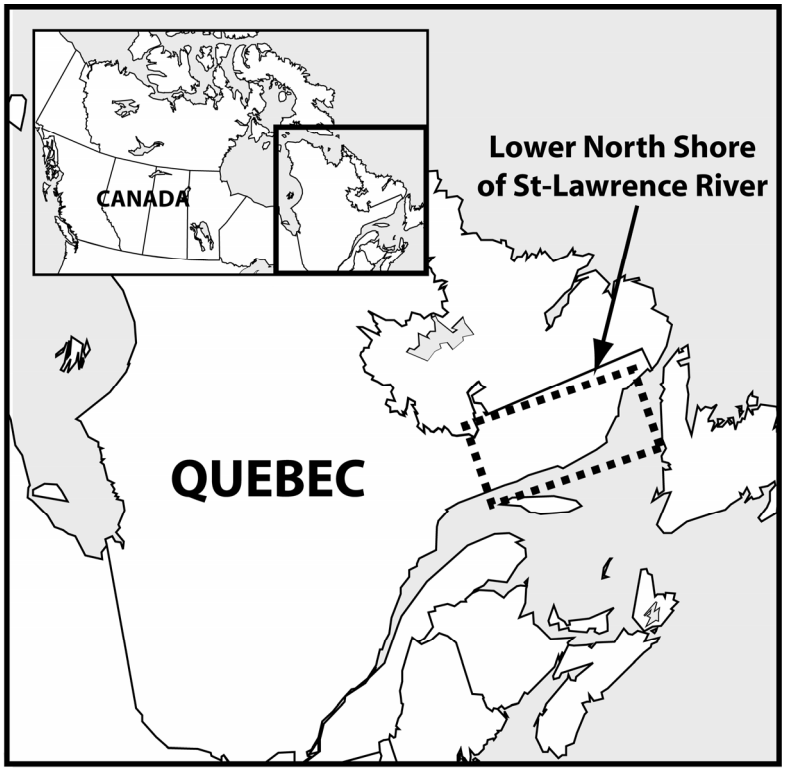

Figure 9.1. Location of the Lower North Shore region in Québec, Canada.

We asked the participants to answer a questionnaire on socio-demographic characteristics and to sign an informed consent form. The questionnaire included information about smoking, previous pregnancies, health problems, ethnic origin and present and past residence. After the delivery, we collected 20 ml of umbilical cord blood, which we centrifuged and froze at –80° C. The vials were sent to the Institut National de Santé Publique du Québec (Quebec City, Canada) every 3 months. We also reviewed the medical chart of the newborns to gather information on the newborns and the deliveries.

Determination of chlorinated compounds levels

Concentrations of 14 PCBs congeners (IUPAC numbers 28, 52, 99, 101, 105, 118, 128, 138, 153, 156, 170, 180, 183 and 187) and of 11 chlorinated pesticides [aldrine, α-chlordane, γ-chlordane, *cis*-nonachlor, *p,p′*-DDE, *p,p′*-DDT, hexachlorobenzene (HCB), mirex, oxychlordane, *trans*-nonachlor and β-hexachlorocyclohexane (BHC)] in blood samples were determined by high-resolution gas chromatography. Plasma samples (2 mL) were extracted, cleaned on florisil columns, taken to a final volume of 100 µL and analysed on an HP-5890 series II gas chromatograph equipped with dual-capillary columns and dual Ni-63 electron-capture detectors (Hewlett-Packard, Palo Alto, CA). Peaks were identified by their relative retention times obtained on the 2 columns, using a computer program developed in-house. The limit of detection was 0.02 µg/L for PCB-congeners and pesticides except for dieldrin, for which the detection limit was 0.1 µg/L. For the sake of simplicity, we have grouped together the 14 PCB congeners, the chlordanes (*trans*-nonachlor, oxychlordane, *cis*-nonachlor, α-chlordane and γ-chlordane), and DDT and DDE (termed ΣPCBs, Σchlordanes, and ΣDDT/DDE, respectively). Because OCs are stored mainly in body fat, all contaminants results are expressed on a lipid basis. We assigned a value of half the detection limit of the method when a contaminant was not detected in a sample. The laboratory methods were rigorously the same for the entire study period.

Determination of blood lipids

Total cholesterol, free cholesterol and triglycerides were measured in plasma samples by standard enzymatic procedures, while phospholipids were determined according to the enzymatic method of Takayama et al. (1977) using a commercial kit (Wako Pure Chemical Industries, Richmond, VA). The concentration of total plasma lipids was estimated according to the formula developed by Phillips et al. (1989).

Determination of n-3 concentrations

To determine the fatty acid composition in plasma phospholipids, 200 µl aliquots of plasma were extracted following the addition of chloroform : methanol (2:1, v/v) in the presence of a known amount of internal standard (diheptadecanoyl phospholipid). Total phospholipids were isolated from the lipid extract by thin-layer chromatography using heptane/isopropyl

ether/acetic acid (60:40:3, v/v/v) as the developing solvent. Following transmethylation, using BF3/methanol, the fatty acids profile was determined by capillary gas-liquid chromatography. The fatty acid composition of plasma phospholipids was expressed as percentages of the total area of all fatty acid peaks from C14:0 to C24:1 (Holub et al. 1987). In this study, plasma phospholipid concentrations of fatty acids correspond to percentages of total fatty acids, by weight.

Statistical analysis

Contaminant concentration variables had log-normal distributions, and were log-transformed for all of the analyses. Therefore, we present all contaminant results as geometric means and confidence intervals. All the other variables had normal distributions, and are presented as arithmetic means ± standard deviation (SD). Of the 403 infants, we included only 392 in the analysis because of missing values (usually caused by insufficient quantity of blood for all the chemical and biochemical analyses).

For the descriptive characteristics of the mothers and the newborns, we used the chi-square (χ^2) statistic to compare proportions and the *t*-test to compare unadjusted means. For temporal trends, we performed multiple regression modeling using the year of birth of the baby (treated as both category and continuous) as the main independent variable. We found a significant association between the region of residence (east or west) and the exposure. We decided to control for this variable because the recruitment of the participants was not equivalent in the two regions every year. We also controlled for the age of the mother (continuous) and the ethnic origin (native or caucasian) because both these characteristics are associated with exposure in this population (Dewailly et al. 1999). The t statistic with Scheffe's correction for multiple comparisons was used to compare adjusted means. Only 12 births occurred late in 1999 and 3 early in 2001. We included these births in the year 2000 in all analyses. The database management and all the statistical analyses were performed with the SAS software (SAS institute, Cary, NC, USA). By convention, we considered $p < 0.05$ significant.

Results

Table 9.1 presents the descriptive characteristics for the 392 mothers and newborns included in the analysis, stratified for ethnic origin. Caucasian women were significantly older and had lower parity than native women. For native women, the proportion of smokers during pregnancy was almost twice that observed for Caucasian women (77.6 % vs. 41.6 %). Native women gave birth to significantly heavier and longer babies than did Caucasian women.

Table 9.1
Descriptive characteristics of the mothers and the newborns

Characteristics	Total Population	Ethnic Origin	
		Caucasians	Natives
Mothers			
Number of participants (%)	392 (100 %)	224 (57.1 %)	168 (42.9 %)
Age (years) [a]	24.8 ± 5.6	26.0 ± 5.3	23.1 ± 5.7 *
Number of previous pregnancies [a]	1.5 ± 1.4	1.1 ± 1.0	2.0 ± 1.8 *
Proportion smoking during pregnancy	57.3 %	41.6 %	77.6 % *
Newborns			
Sex (proportion of male)	50.2 %	51.8 %	48.2 %
Weight (kg) [a]	3.49 ± 0.50	3.40 ± 0.47	3.61 ± 0.52 *
Length (cm) [a]	51.8 ± 2.3	51.5 ± 2.3	52.2 ± 2.1 *

[a] Arithmetic means ± standard deviation. * Significantly different compared to the caucasians (χ^2 test for proportions and t test for means, $p < 0.05$).

Table 9.2 presents the adjusted mean concentrations of contaminants in cord blood according to the year of birth of the baby. These results show a steady decrease of the mean concentrations for all groups of contaminants between 1993 and 2000. Between 1993 and 2000, the unadjusted mean levels of ΣPCBs, Σchlordanes, ΣDDT/DDE and HCB in cord blood decreased by 63.1 %, 25.2 %, 65.7 %, and 69.4 % respectively ($p < 0.0001$). When adjusted for ethnicity, age of the mother, and region of residence, the decreases observed were slightly lower (50.6 %, 21.0 %, 55.4 % and 65.1 % for ΣPCBs, Σchlordanes, ΣDDT/DDE and HCB, respectively, $p < 0.0001$). No significant seasonal or monthly pattern was detected.

Table 9.2
Adjusted mean concentrations [a] (and 95 % confidence interval) of contaminants in cord blood by the year of birth

Year of birth	ΣPCBs (µg/kg)	Σchlordane (µg/kg)	ΣDDT/DDE (µg/kg)	HCB (µg/kg)
1993 (n = 62)	345.2 (301.5 – 395.2)	32.9 (30.7 – 35.3)	295.7 (264.0 – 331.3)	35.5 (32.0 – 39.2)
1994 (n = 62)	252.5 (220.5 – 289.2)	29.6 (27.6 – 31.7)	226.1 (201.7 – 253.3)	19.2 (17.3 – 21.2) *
1995 (n = 82)	243.5 (216.4 – 274.2) *	28.6 (26.9 – 30.4)	208.1 (188.5 – 229.9) *	17.9 (16.3 – 19.5) *
1996 (n = 71)	222.5 (195.9 – 252.8) *	28.1 (26.4 – 30.0)	182.4 (163.9 – 203.0) *	16.9 (15.4 – 18.6) *
1997 (n = 50)	188.5 (162.1 – 219.2) *	25.9 (24.0 – 28.0) *	154.6 (136.3 – 175.5) **	13.3 (11.9 – 14.9) #
2000 (n = 65)	153.5 (134.4 – 175.4) ##	25.8 (24.1 – 27.6) *	119.1 (106.6 – 133.2) ##	11.6 (10.5 – 12.8) ##

[a] Geometric means on a lipid basis adjusted for ethnic origin, region of residence and age.
*Concentration significantly different from that of 1993. **Concentration significantly different from that of 1993 and 1994. #Concentration significantly different from that of 1993, 1994, and 1995. ##Concentration significantly different from that of 1993, 1994, 1995, and 1996; *t*-test on adjusted means with Scheffe's correction for multiple comparisons.

We calculated the adjusted annual decreases by multiple regression, treating the year of birth as a continuous variable. Table 9.3 presents these results stratified by ethnic origin. The trends for ΣPCBs, Σchlordanes, ΣDDT/DDE, and HCB for both ethnic origins were strongly significant ($p < 0.0001$). The only exception to this was the trend of the chlordane concentrations in the Caucasian population, which was still significant but less than the other trends ($p = 0.009$). Because the calculated trends were linear using log-transformed dependant variables (contaminant concentrations), our results denote an exponential decrease of the concentrations of contaminants in cord blood between 1993 and 2000. When we analyzed PCB congeners separately, we observed the strongest in the most abundant congeners. In fact, all the non-significant trends we observed were for congeners for which > 50 % of the samples were below the limit of detection. The annual decrease for the sum of the PCBs congeners increased from 10.0 % to 12.2 % when we excluded the least abundant congeners (detected in less than 50 % of the samples). We observed little variation between trends according to the ethnic origin. Most of the differences were non-significant, but PCB congeners 99, 105, 156 and 183 showed a significantly steeper trend for natives than for Caucasians. When we stratified the data by the age of the mother, the downward trends were similar in every age group (data not shown).

The proportion of n-3 fatty acids in the blood was examined as a surrogate of long-term fish consumption (Silverman et al. 1990). We found a significant correlation between n-3

fatty acids and OC concentrations for Natives, but not for Caucasians (results not shown). We also found a slight nonsignificant decrease in n-3 proportions between 1993 and 2000 in Natives. As shown in Table 9.4, the small variation in n-3 could not explain the diminution of OC concentrations. This indicates that a potential fish consumption diminution was only slightly responsible for the observed trends.

Table 9.3

Adjusted annual decreases (%)[a] (and 95 % confidence interval) of contaminants in cord blood between 1993 and 2000

Contaminants	Total population (n = 392)	Ethnic origin	
		Caucasians (n = 224)	Natives (n = 168)
Σ of chlordanes	3.1 % (1.9 – 4.3 %)**	2.2 % (0.6 – 3.8 %)*	4.1 % (2.3 – 5.8 %)**
Σ of DDT and DDE	11.6 % (9.8 – 13.4 %)**	10.8 % (8.2 – 13.4 %)**	12.4 % (10.1 – 14.7 %)**
Hexachlorobenzene	12.5 % (10.8 – 14.2 %)**	12.3 % (9.9 – 14.6 %)**	12.6 % (10.0 – 15.0 %)**
Σ of the 14 PCB congeners	10.0 % (7.8 – 12.2 %)**	9.3 % (6.2 – 12.3 %)**	10.6 % (7.6 – 13.5 %)**
PCB congener 28 (4.9 %)[c]	0.3 % (-1.3 – 1.8 %)	-0.5 %[b] (-2.3 – 1.3 %)	1.2 % (-1.6 – 4.0 %)
PCB congener 52 (13.3 %)	0.2 % (-1.4 – 1.7 %)	0.4 % (-1.9 – 2.8 %)	-0.1 %[b] (-1.8 – 1.3 %)
PCB congener 99 (73.3 %)	13.8 % (11.6 – 16.0 %)**	12.0 % (8.8 – 15.0 %)**	15.8 % (12.7 – 18.8 %)**
PCB congener 101 (17.9 %)	6.5 % (4.6 – 8.48 %)**	5.5 % (3.1 – 7.8 %)**	8.0 % (4.8 – 11.1 %)**
PCB congener 105 (29.1 %)	5.0 % (3.0 – 6.9 %)**	1.3 % (-0.9 – 3.5 %)	8.8 % (5.8 – 11.8 %)**
PCB congener 118 (78.6 %)	13.1 % (10.5 – 15.5 %)**	11.6 % (8.0 – 15.0 %)**	14.3 % (10.8 – 17.7 %)**
PCB congener 128 (2.0 %)	-0.6 %[b] (-1.5 – 0.3 %)	-1.6 %[b] (-2.6 – -0.5 %)*	0.8 % (-0.8 – 2.3 %)
PCB congener 138 (96.4 %)	12.9 % (10.2 – 15.4 %)**	13.3 % (9.5 – 16.7 %)**	12.0 % (8.6 – 15.3 %)**
PCB congener 153 (97.7 %)	12.2 % (9.4 – 14.9 %)**	13.4 % (9.3 – 17.2 %)**	10.5 % (6.9 – 13.9 %)**
PCB congener 156 (46.7 %)	4.9 % (2.7 – 7.0 %)**	2.7 % (-0.2 – 5.5 %)	7.0 % (4.0 – 9.9 %)**
PCB congener 170 (58.2 %)	9.6 % (7.1 – 12.1 %)**	8.2 % (4.7 – 11.6 %)**	10.9 % (7.5 – 14.1 %)**
PCB congener 180 (88.8 %)	12.1 % (9.3 – 14.8 %)**	12.0 % (8.0 – 15.9 %)**	11.9 % (8.3 – 15.5 %)**
PCB congener 183 (36.5 %)	3.6 % (1.6 – 5.5 %)**	1.4 % (-1.0 – 3.7 %)	5.8 % (2.8 – 8.7 %)**
PCB congener 187 (75.0 %)	11.0 % (8.5 – 13.4 %)**	10.5 % (6.9 – 13.8 %)**	11.3 % (7.9 – 14.6 %)**

[a] Percentage of decrease per year calculated by multiple regression in which the year of birth is a continuous variable. The slope is adjusted for the region of residence, the age of the mother and the ethnic origin (when applicable). Since the dependant variable (contaminants concentrations) of the regression was logarithmically transformed, each annual decrease and confidence interval were calculated from de slope (β) of the regression estimate (and its confidence interval) according to the following expression : $[100 \times (1 - e^{\beta})]$.

[b] A negative value denotes an increase, rather than a decrease.

[c] Percentage of the samples above the limit of detection.

* $p < 0.01$; ** $p < 0.001$.

Table 9.4

Total decrease of OCs in cord blood between 1993 and 2000 attributed to n-3 fatty acids

Contaminants	Adjusted* total decrease between 1993 and 2000		Adjusted* decrease explained by n-3 between 1993 and 2000	
	Caucasians	Natives	Caucasians	Natives
Σ of chlordanes	15.9 %	23.9 %	0.9 %	1.4 %
Σ of DDT and DDE	53.3 %	55.8 %	1.6 %	0.9 %
Hexachlorobenzene	66.8 %	60.4 %	2.0 %	0.4 %
Σ of the 14 PCB congeners	47.7 %	52.5 %	0.7 %	2.2 %

* The differences between mean level of 1993 and 2000 were adjusted for the region of residence and the age of the mother.

Discussion

The results of this study underline a strong exponential downward trend of prenatal exposure to PCBs and chlorinated pesticides. The trends were observed for all the examined contaminants, in both ethnic groups and in all age groups. The substances considered in this study are all recognized persistent pollutants and their use is prohibited or severely restricted in Canada. Despite a very long half-life, the reduction of the production and release of these OCs in the last 10-20 years is expected to result in a decrease of the burden in the environment, in wild animal and, ultimately, in humans.

The ubiquitous decrease in OC burden suggests that the generalized reduction in OCs in wild caught fishes and animals might have played an important role. As noted in the introduction, a decrease of PCBs and DDT in wild animals and fish has already been observed (Muir et al. 1999, Muir et al. 2000, Schmitt et al. 1999). The population targeted in this study is exposed to OCs mainly through seabird egg consumption (Dewailly et al. 1992). During the last 10 years, PCBs, HCB, and DDE levels in herring gull eggs sampled in the Lower North Shore region have dropped by more than 85 % (N. Burgess, unpublished results). This decrease has most likely played a key role in the trends observed in this study. A similar decrease was also observed in herring gull eggs from the Great Lakes in the 1980s and early 1990s (Hebert et al. 1994, Ryckman et al. 1994).

We cannot, however, rule out the influence of a modification of dietary habits. The proportion of n-3 fatty acids in blood reflects the long-term fish consumption of an individual (Silverman et al. 1990). In our study, n-3 fatty acids did not show any significant time trend and could not explain the decrease observed in OCs. Reduction of fish consumption is thus unlikely to have contributed to the decrease of OCs in cord blood. However, a modification of seabird egg consumption could have greatly influenced the OC exposure of the mothers and, subsequently, of the newborns without affecting the proportion of n-3 in blood. Awareness of the level of contamination of specific species or types of tissues might have been involved. In 1996, a report from the public health board of the Lower North Shore region stated that a decrease of seabird egg consumption had been observed between 1990 and 1995 (Cartier 1996). Furthermore, many efforts have been

made to inform this population of the contamination of sea-bird eggs and of the potential effects of OCs of human health. A complete dietary study would be needed to better associate dietary changes with OC exposure.

The time trends of DDT/DDE and HCB concentrations observed in this study are similar to trends in breast milk found by Noren and Meironyte (2000). The trend for PCBs, however, was twice as strong in our study. Although the populations, the time intervals, and the analytical methods varied, the fact that similar trends were observed in two populations of mothers with different culture, lifestyle and diet supports the hypothesis that a generalized reduction of OC concentration in the environment has played a role in the diminution of OCs in our population.

To our knowledge, this is the first study evaluating the time trend of OC prenatal exposure. The fetus is particularly vulnerable to xenobiotics, and close monitoring of the populations at risk for high exposure is essential. This study shows that the situation has greatly improved in the last years. Prenatal exposure has diminished, and exposure through breast milk will most likely be decreased as well.

Acknowledgments

We are indebted to Germain Lebel for database management, to J.-P. Weber and É. Pelletier (Quebec toxicology Center) for OCs analyses, to B. Holub for lipid analyses, and to the staff of Sept-Iles hospital and Blanc Sablon hospital for their useful assistance. We thank the Saint-Laurent Vision 2000 program for financial support. F. D. is supported by the Canadian Institutes of Health Research.

Annexe 2 – Les concentrations de vitamine A dans le sang de cordon ombilical d'enfants de trois régions du Québec

Dallaire F., Dewailly E., Shademani R., Laliberté C., Bruneau S., Rhainds M., Blanchet C., Lefebvre M., Ayotte P., 2003. Vitamin A concentration in umbilical cord blood of infants from three separate regions of the province of Québec (Canada). *Can J Public Health* 94(5):386-90.

Résumé

Contexte: Les femmes inuites du Nord du Québec consomment des quantités insuffisantes de vitamine A. Cette étude a été entreprise pour évaluer la prévalence de la déficience en vitamine A chez les nouveau-nés de trois populations distinctes de la province de Québec. **Méthodes**: 594 nouveau-nés ont été recrutés pour cette étude (375 nouveau-nés inuits du Nord du Québec (Nunavik), 107 nouveau-nés caucasiens et autochtones de la Basse-Cote-Nord du Saint-Laurent (BCN) et 112 nouveau-nés du Sud du Québec où la déficience en vitamine A est peu fréquente. Les mères étaient recrutées lors de l'accouchement et la vitamine A (rétinol) était dosée dans le sang de cordon ombilical par chromatographie liquide à haute pression en phase inversée. **Résultats**: Les nouveau-nés du Nunavik et de la BCN avaient une concentration moyenne de vitamine A significativement plus basse que dans le Sud du Québec (15,7 µg/dL, 16,8 µg/dL et 20,4 µg/dL respectivement). Les différences observées étaient semblables en ajustant pour le sexe de l'enfant et le poids à la naissance. Les résultats ont aussi montré que 8,5 % de nouveau-nés du Nunavik et 12,2 % des nouveau-nés de la BCN avaient une concentration inférieure à 10,0 µg/dL, un niveau que l'on croit être indicatif d'une déficience néonatale en vitamine A. **Conclusion**: Ces données suggèrent qu'un programme de supplémentation bien planifié en vitamine A visant les femmes enceintes du Nunavik et de la BCN pourrait être nécessaire.

Abstract

Background: Inuit women from Northern Québec have been shown to consume inadequate quantities of vitamin A. This study was conducted to evaluate the prevalence of blood

vitamin A deficiency in newborns from 3 distinct populations of the province of Québec. **Methods**: 594 newborns were included in this study (375 Inuit newborns from northern Québec (Nunavik), 107 Caucasian and Native newborns from the Lower Northern Shore of the Saint-Lawrence River (LNS) and 112 newborns from Southern Québec where clinical vitamin A deficiency is uncommon). Mothers were recruited at delivery and vitamin A (retinol) was analyzed from umbilical cord blood samples by reversed-phase high-pressure liquid chromatography. **Results**: Nunavik and LNS newborns had significantly lower mean vitamin A concentrations in cord blood compared to Southern Québec participants (15.7 µg/dL, 16.8 µg/dL and 20.4 µg/dL respectively). The differences observed were similar when adjusted for sex and birth weight. Results also showed that 8.5 % of Nunavik newborns and 12.2 % of LNS newborns were below 10.0 µg/dL, a level thought to be indicative of blood vitamin A deficiency in neonates. **Conclusion**: These data suggest that a carefully planned vitamin A supplementation program during pregnancy in Nunavik and LNS might be indicated to promote healthy infant development

Introduction

Vitamin A influences the expression of many genes and is essential for normal growth. Its involvement in infant development, vision and cell differentiation is well known. Adequate maternal stores of vitamin A are essential during pregnancy to meet the needs of the fetus. Vitamin A deficiency during development and early childhood delays growth, impairs vision and increases the severity of infections, ultimately leading to many otherwise preventable deaths (Dudley et al. 1997, Humphrey et al. 1992, Semba 1994, Underwood 1994).

Vitamin A deficiency among children is an important public health problem primarily in developing countries (Humphrey et al. 1992, Villamor & Fawzi 2000). In Canada, Inuit infants are also at risk of vitamin A deficiency because Inuit women of childbearing age have been shown to consume inadequate quantities of vitamin A (Blanchet et al. 2000, Kuhnlein et al. 1996, Lawn et al. 1998). Canadian Inuit preschool children have been shown to have a high incidence of infections (Dufour 1988, Proulx 1988). It is therefore important to determine if vitamin A deficiency, an important risk factor for infectious

diseases, is prevalent in this population. This study attempts to document the prevalence of blood vitamin A deficiency in Inuit neonates. Comparison of vitamin A concentrations between studies has proven difficult because of variations in analytical methods. In this study, we compare the vitamin A concentrations in umbilical cord serum samples of Inuit neonates from the northern part of the province of Québec (Nunavik) with cord blood levels of two different populations from Québec, with all samples being analyzed in the same laboratory.

Methods

Populations and recruitment

The participants in this study came from three different populations in the province of Québec (Canada). They were originally recruited for the evaluation of prenatal exposure to food-chain contaminants (Dewailly et al. 1998a, Dewailly et al. 1999, Rhainds et al. 1999). In each population, a subset of the participants also agreed to participate in the present study. The first population was composed of mothers and their newborns from the 14 small Inuit communities of Nunavik. Nunavik is a vast and remote region situated in the northernmost region of the province of Québec (Figure 10.1). Between 1993 and 1996, women were recruited when they arrived for delivery at one of the two regional health centres. Four hundred and ninety-one women accepted to participate in the contaminants study and of these, 419 women (85.3 %) were randomly selected to participate in the present study.

The second population was recruited in the Lower North Shore (LNS) region of the St-Lawrence River. This region is comprised of 15 villages and is remote and isolated from the rest of the province of Québec (Figure 10.1). About 45% of the inhabitants of the LNS are Aboriginal (mainly Montagnais Indians). As in Nunavik, women were recruited when they arrived for delivery at one of the two regional health centers of the LNS. The recruitment was done from 1993 to 1997 and 467 women accepted to participate in the contaminants study. Of these, 108 (23.1 %) were randomly selected for the present study.

The third population is composed of women admitted for delivery in one of the 10 participating hospitals from 10 administrative regions in the province of Québec. These hospitals cover regions thought to be representative of the southern portion of the province of Québec where clinical vitamin A deficiency is uncommon. Women were asked to participate when they arrived at one of the hospitals for delivery. Between 1993 and 1995, a total of 1109 women accepted to participate in the contaminants study and 112 (10.1 %) were randomly selected to participate in the present study.

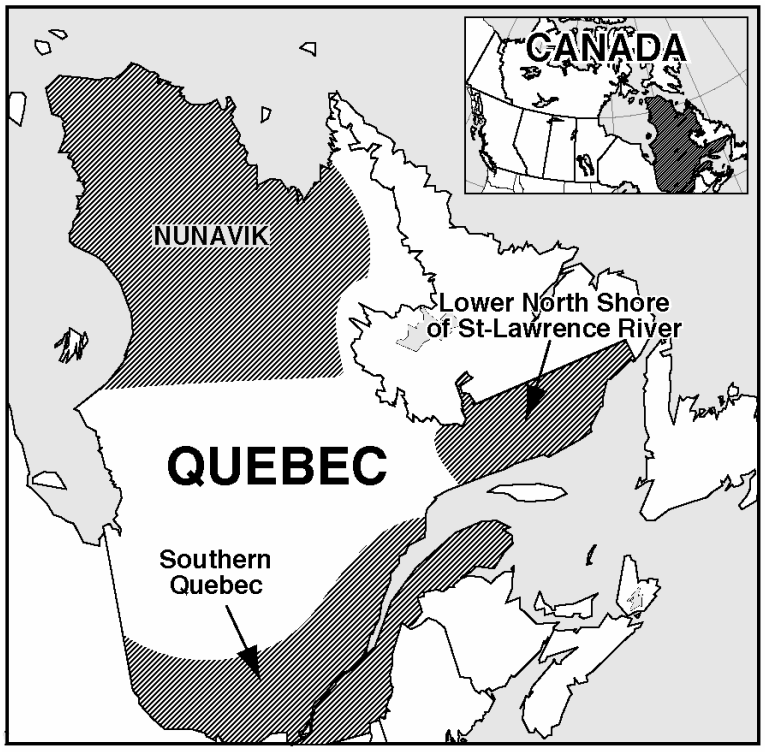

Figure 10.1 - Location of Nunavik, Lower North Shore of the Saint-Lawrence River and Southern Quebec

Data collection and sample analysis

After signing the appropriate consent forms, all mothers answered a short questionnaire relating to their sociodemographic characteristics. Data on the newborns (weight, gestational age, APGAR, etc.) were gathered from the medical files. After delivery, 15 to 20 ml of cord blood were sampled by venipuncture, centrifuged and then frozen at –80°C. The samples were then sent to the Institut National de Santé Publique du Québec (Québec City, Canada) for vitamin A (retinol) analysis. They were stored 1 to 3 months before being

processed. Ethanol was added to the samples to denature proteins, and retinol was extracted from the solution with hexane. After concentration, retinol was redissolved in ethanol. Retinol concentration was then determined by reversed-phase high-pressure liquid chromatography using Waters pumps (model 600), a programmable diode array detector (model 996) and Waters uBondapaq C18 columns with retinal acetate as the internal standard (Catignani & Bieri 1983, MacCrehan & Schonberger 1987, Nierenberg & Lester 1985). The laboratory technicians were "blinded" to the region of origin of the samples and the samples were processed in a random order. This project was approved by the ethics committee of the Laval University medical research centre.

Statistical analysis

Arithmetic means ± standard deviations are shown. We used Scheffe's test for multiple comparisons to compare unadjusted means among the 3 populations. We compared proportions using the χ^2 statistic. We also performed multivariate regression to control for birth weight and sex. We controlled for birth weight rather than gestational age since birthweight was more closely associated with vitamin A concentrations in cord blood. We used the t-test in multivariate linear regression to compare adjusted means (with Scheffe's adjustment for multiple comparisons when necessary). All statistical analyses were performed with the SAS software (SAS Institute, Cary, NC, USA). By convention, a $P < 0.05$ was considered significant.

Results

A total of 639 women/infant pairs were recruited for the present study. Due to missing values (insufficient blood sample, sociodemographic questionnaire not completed), we excluded 45 participants (6.9 %) from the statistical analysis. Table 10.1 shows the characteristics of the 594 mother/infant pairs included in the analysis according to their region of residence. Mothers from Nunavik were younger than their counterparts from Southern Québec. They also had more previous pregnancies than the mothers from LNS and Southern Québec. As expected, the ethnic origins were considerably different according to the region of residence. Nunavik participants were exclusively Inuit while 51% of the mothers from LNS were Caucasians and 49% were Aboriginal. The Southern

Québec region was comprised of mostly Caucasians with a few mothers with other ethnic origins. Women from LNS gave birth to slightly heavier babies but this difference was not significant. Maternal smoking during pregnancy was much more prevalent in Nunavik (85.9 %) than in the LNS (50.0 %) and in Southern Québec (28.6 %). Maternal smoking, maternal age and the number of previous pregnancies did not significantly affect the vitamin A levels in either region (data not shown) and were thus excluded from the multivariate analyses.

Table 10.1
Characteristics of participating mothers and their infants according to region of residence

Characteristics	Nunavik (n = 375)	Lower North Shore (n = 107)	Southern Québec (n = 112)
Mothers			
Age (years)*	23.7 ± 5.3 [a]	24.4 ± 5.3 [a]	28.6 ± 4.5 [b]
Number of previous pregnancies*	3.7 ± 2.2 [a]	1.5 ± 1.6 [b]	1.2 ± 1.1 [b]
Ethnic origin [f]			
Caucasian (%)	0 %	51.4 %	95.5 %
Inuit (%)	100 %	0 %	0 %
Aboriginal other than Inuit (%)	0 %	48.6 %	0.9 %
Other (%)	0 %	0 %	3.6 %
Smoking during pregnancy (%) †	85.9 % [c]	50.0 % [d]	28.6 % [e]
Infants			
Sex (% of males)	46.9 % [c]	48.6 % [c]	59.8 % [d]
Birthweight (g)*	3491.3 ± 453.4	3537.6 ± 535.2	3439.9 ± 466.1
Gestational age (y)*	39.0 ± 1.4	39.5 ± 1.3	39.6 ± 1.3

[a, b] Means with different letters are significantly different (Scheffe's test for multiple comparisons; $\alpha = 0.05$).
[c, d, e] Proportions with different letters are significantly different (χ^2; $p < 0.05$).
[f] The ethnic distribution is significantly different in every region ($p < 0.05$).
* Means ± standard deviations.
† Information on 59 subjects was missing for smoking during pregnancy.

The concentrations of vitamin A in cord serum were normally distributed in each region with a slightly lower standard deviation in Nunavik. Table 10.2 presents the mean vitamin A concentrations and the quartiles boundaries for each region. The mean concentration of vitamin A in Nunavik was 15.7 ± 4.9 µg/dL compared to 16.8 ± 5.6 µg/dL in LNS and 20.4 ± 5.8 µg/dL in Southern Québec. The differences were significant between Nunavik and Southern Québec and between LNS and Southern Québec. In LNS, the newborns with Aboriginal origins had a slightly higher mean vitamin A concentration than the Caucasians newborns (17.5 µg/dL vs. 16.2 µg/dL), but the difference was not significant (P = 0.21).

Data from newborns of both ethnic origins in LNS were therefore merged in the remaining analyses.

Table 10.2
Vitamin A concentrations in cord blood according to region of residence

Region	Mean	CI 95 %	5th percentile	25th percentile	Median	75th percentile	95th percentile
	µg/dL	µg/dL	µg/dL	µg/dL	µg/dL	µg/dL	µg/dL
Nunavik	15.7 ± 4.9 [a]	15.2 – 16.2	8.8	12.2	15.2	18.2	24.3
Lower North Shore	16.8 ± 5.6 [a]	15.8 – 17.9	7.7	12.9	17.2	20.6	25.1
Southern Québec	20.4 ± 5.8 [b]	19.3 – 21.5	11.4	16.2	19.5	24.4	29.8

CI 95 % = 95% confidence interval of the mean.
[a, b] Means with different letters are significantly different (Scheffe's test from multiple comparisons; $\alpha = 0.05$).

In Nunavik, 47.5 % of the samples were below 15.0 µg/dL (table 10.3). This proportion was higher than that observed in LNS (38.3 %, P = 0.06) and in Southern Québec (14.3 %, P < 0.0001). As much as 8.5 % of the samples in Nunavik fell below 10.0 µg/dL (table 10.3). This proportion was slightly lower as compared to that of LNS (12.2 %, P = 0.46) but was significantly higher than that in Southern Québec (2.7 %, P = 0.02).

Table 10.3
Proportions of infants in the different cord serum vitamin A categories according to region of residence

Region	Proportion of the population in each vitamin A category					global χ^2 P values
	Less than 10.0 µg/dL	10.0 – 14.9 µg/dL	15.0 – 19.9 µg/dL	20.0 – 24.9 µg/dL	25.0 µg/dL and more	
Nunavik	8.5 %	38.3 %	35.5 %	13.7 %	4.0 %	Each region is significantly different than the two others (P < 0.01)
Lower North Shore	12.2 %	26.2 %	29.9 %	26.2 %	5.6 %	
Southern Québec	2.7 %	11.6 %	41.1 %	21.4 %	23.2 %	

Table 10.4 shows the mean concentrations of vitamin A among the regions adjusted for sex and birth weight using multivariate regression. The mean vitamin A concentration in Nunavik adjusted for sex and birth weight was significantly lower than the mean concentration in Southern Québec (15.6 µg/dL vs. 20.7 µg/dL, P < 0.0001). The adjusted difference was higher than the unadjusted difference (5.1 µg/dL vs. 4.7 µg/dL) principally because of the confounding effect of sex. The adjusted mean concentration in Nunavik was slightly lower than in LNS (15.6 µg/dL vs. 16.7 µg/dL) but the difference was not significant (P = 0.15). Table 10.4 also shows the independent effects of sex and birthweight

on vitamin A in cord serum. Females had higher concentrations of vitamin A than males, especially in Nunavik. Birthweight was also positively associated with vitamin A in all three groups.

Table 10.4
Adjusted means* and confidence intervals of vitamin A in cord serum according to sex and birth weight for each region of residence

Determinants	Nunavik (µg/dL)			Lower North Shore (µg/dL)			Southern Québec (µg/dL)		
All infants	15.6	(15.1 – 16.1) [a]		16.7	(15.7 – 17.6) [a]		20.7	(19.8 – 21.7) [b]	
Sex									
Male	14.5	(13.8 – 15.2)	P< 0.01	16.3	(14.8 – 17.8)	P = 0.32	19.6	(18.2 – 20.9)	P = 0.06
Female	16.8	(16.1 – 17.4)		17.4	(15.9 – 18.8)		21.6	(20.0 – 23.3)	
Birth weight									
Vitamin A level Increase for each 100 g increase in birth weight	3.06	(2.01 – 4.11)	P < 0.01	2.91	(0.95 – 4.87)	P < 0.01	2.99	(0.71 – 5.26)	P = 0.01

* Means are adjusted for birth weight and sex using multivariate linear regression.
[a, b] Means with different letters are significantly different (t test on adjusted means with Scheffe's adjustment from multiple comparisons; $\alpha = 0.05$).

Discussion

In this study, we found that newborns from Nunavik and the Lower North Shore of the St-Lawrence River had lower neonatal concentrations of vitamin A than their counterparts from regions of Southern Québec. Mean concentrations of vitamin A in cord blood vary widely among studies and populations. Other studies have reported similar concentrations (Chan et al. 1993, Shirali et al. 1989, Yeum et al. 1998), lower concentrations (Tolba et al. 1998) and higher concentrations (Ghebremeskel et al. 1994, Godel et al. 1996, Lindblad et al. 1998, Neel & Alvarez 1990) in cord blood of healthy term newborns as compared to those presented here. Many factors can affect the comparability among studies. In this study, to increase the comparability among the different regions, the same laboratory processed all the samples and used the same analytical method. We also controlled for sex and birth weight in the statistical analysis since both of these factors were associated with vitamin A concentration at birth.

Lifestyle and nutrition are likely to be responsible for the lower vitamin A concentrations seen in Nunavik. Blanchet et al. (2000) investigated the diet of Inuit women from Nunavik and found that their daily intake of vitamin A fell below the recommended intake level. Vitamin-A rich traditional food items such as ringed seal liver were consumed infrequently and contributed little to the vitamin A intake over the entire year (Blanchet et al. 2000, Kuhnlein & Soueida 1992, Kuhnlein et al. 1996). Fruits and vegetables are less available and often more expensive in Nunavik communities as compared to Southern Québec and are therefore consumed less frequently in northern regions. Inuit women from other regions of northern Canada have also been shown to have a vitamin A-deficient diet (Kuhnlein et al. 1996, Lawn et al. 1998). Newborns from LNS had similar cord blood vitamin A concentrations to the Nunavik newborns. As in Nunavik, inhabitants from LNS are isolated, live in harsh climatic conditions and reside in small coastal communities. Because of this, as well as other cultural and traditional reasons, their main source of food comes from the sea. Fruits, vegetables and fortified milk contribute less to their daily diet than these country food items.

The region of Nunavik is inhabited almost exclusively by Inuit while the Southern Québec participants were mainly of Caucasian descent. One could speculate that race and genetic factors could be responsible for the differences observed here. Godel et al. (1996) observed lower concentrations of vitamin A in cord plasma among Inuit newborns as compared to Caucasian newborns in a northern Canadian population. The concentrations observed were higher than the ones in this study but the difference between Inuit and Caucasians was similar. Based on a food frequency questionnaire, the authors concluded that the difference was most likely due to environmental and nutritional factors rather than ethnic origin itself. Looker et al. (1988) studied the differences in vitamin A status between Mexican-Americans, non-Hispanic blacks and whites in the U.S. They too concluded that nutrition and lifestyle factors explained most of the differences they observed. We believe that in the present study, the differences in vitamin A concentrations observed among the regions are due mostly to nutritional characteristics rather than race.

Data on the supplementation of vitamin A during pregnancy in these three populations are scarce. Multivitamin tablets containing vitamin A are often recommended during pregnancy but the extent of the implementation of this measure among these populations is not known. Knowing, however, that compliance is often problematic in Nunavik, it is likely that part of the difference in the vitamin A levels observed in this study was due to some variation in multivitamin supplement intake among the participating populations.

There is no clear consensus on the "cut-off concentration" for vitamin A deficiency in cord blood. Godel et al. (1996) suggested that the cutoff for vitamin A deficiency should be the same for both adults and newborns. Our results from Southern Québec, where malnutrition and vitamin A clinical deficiency are rare, suggest that vitamin A concentrations among healthy babies can be well below the normal adult standard of 20 µg/dl. Several studies have shown that cord plasma vitamin A concentrations in non-deficient populations averaged about 50 % of values in maternal plasma (Baker et al. 1975, Oostenbrug et al. 1998, Rondo et al. 1995, Shirali et al. 1989, Yeum et al. 1998). Linblad et al. (1998) also observed a clear relationship between age and vitamin A concentration in the first two years of life and suggested a cut-off concentration for deficiency of 10 µg/dl for one-month-old infants. These studies and our results suggest that a cut-off value of 10 µg/dl in cord blood is appropriate for the detection of vitamin A deficiency at birth. Therefore, according to this value, as many as 8.5 % of the Nunavik infants and 12.2 % of the LNS newborns included in our study were born with deficient concentrations of circulating vitamin A.

This study shows that the prevalence of blood vitamin A deficiency is high in Nunavik and LNS and could affect as many as one in ten newborns. Since vitamin A deficiency is associated with important health problems, studies addressing clinical deficiency should be initiated to determine if a supplementation program during pregnancy should be considered by public health officials.

Acknowledgements

Recruitment and analysis were financed through the Northern Contaminants Program (Indian and Northern Affairs Canada), the St-Laurent Vision 2000 program (Health Canada and Environnement Québec), and by Hydro Québec. The authors wish to thank the staff of

the participating hospitals and health centres for their helpful collaboration. F. Dallaire is supported by the Canadian Institutes for Health Research.

Annexe 3 – Impact d'une déficience néonatal en vitamine A sur l'incidence d'infections respiratoires chez les enfants du Nunavik

Cameron C., Dallaire F., Vézina C., Muckle G., Bruneau S., Ayotte P., Dewailly E., 2004. Vitamin A deficiency in cord blood and its impact on acute respiratory infections among preschool Inuit children. En preparation.

Résumé

Objectif: Évaluer si la concentration de vitamine A dans le sang de cordon ombilical est associée à l'incidence d'infections respiratoires aiguës chez les enfants inuits d'âge préscolaire au Nunavik. **Devis d'étude:** Les dossiers médicaux de 305 enfants ont été revus pour une période couvrant les cinq premières années de vie. L'association entre la vitamine A dans le sang de cordon ombilical et l'incidence d'otites moyennes aiguës (OMA), d'infections des voies respiratoires supérieures (IVRS) et inférieures (IVRI) et d'hospitilasation pour une IVRI a été évaluée à l'aide de la régression de Poisson. **Résultats**: Comparativement aux enfants ayant une concentration de vitamine A ≥ 20 µg/dl, les rapport de taux d'incidence ajustée (RT) pour les enfants avec < 20 µg/dl s'étendaient de 1,06 à 1,62 pour les OMA, de 1,12 à 1,34 pour les IVRI et de 1,09 à 1,43 pour les hospitalisations pour une IVRI. La plupart de RT étaient statisitquement significatifs pour les OMA et les IVRI, mais pas pour les hospitalisations pour une IVRI. Aucune association n'a été détectée pour les IVRS. **Conclusion**: Une déficience néonatale en vitamine A serait un facteur de risque significatif pour l'incidence d'OMA et d'IVRI dans cette population.

Abstract

Objective: To assess if vitamin A concentration in umbilical cord blood is associated with incidence and severity of respiratory infections in preschool Inuit children from Nunavik. **Study design:** The medical charts of 305 children were reviewed from 0 to 5 years of age. The association between vitamin A concentration in umbilical cord plasma and the incidence rates of acute otitis media (AOM), upper and lower respiratory tract infections (URTIs and LRTIs) and hospitalization rates for LRTIs was evaluated using Poisson regression. **Results**: Compared to children with vitamin A concentration ≥ 20 µg/dl,

adjusted rate ratios (RR) for children below 20 µg/dl ranged between 1.06-1.62 for AOM, 1.12-1.34 for LRTIs, and 1.09-1.43 for hospitalization for LRTIs. Most RRs were statistically significant for AOM and LRTIs, but not for hospitalization for LRTIs. No association was found for URTIs. **Conclusion**: Neonatal vitamin A deficiency appears to be a significant risk factors for AOM and LRTIs in this population.

Introduction

Vitamin A plays a major role in growth, development and vision (Sommer 1997, West et al. 1989). Vitamin A deficiency is well known to impair resistance to infection, especially in early age (Bhaskaram 2002, Kapil & Bhavna 2002, Ross 1996, Ross & Stephensen 1996, Semba 1994, West et al. 1989) and many authors have identified increased rates or augmented severity of infection in vitamin A deficient children (Basu et al. 2003, Bloem et al. 1990, Dudley et al. 1997, Pinnock et al. 1986, Sommer 1990, Sommer et al. 1984). Insufficient vitamin A intake has typically been described in developing countries. However, our group recently showed that a significant proportion of Inuit infants from Nunavik (Northern Québec, Canada) were born with low blood levels of vitamin A (Dallaire et al. 2003).

The Nunavik region is located in the northernmost part of the province of Québec. Around 9 600 Inuit inhabit 14 Inuit communities spread out on the Coast line of the Hudson Bay, the Hudson Strait, and the Ungava Bay. Children from Nunavik are burdened by a high rate of acute infections (unpublished results), a situation similar to other Inuit communities throughout the world (Banerji et al. 2001, Davidson et al. 1994, Koch et al. 2002, Wainwright 1996).

This study is the second phase of a previous study that evaluated vitamin A concentration in cord blood of newborns from three regions of the province of Québec (Dallaire et al. 2003). Before considering the implementation of a supplementation program, it was important to evaluate the potential impact of low vitamin A concentration at birth on infection incidence in the first years of life. Thus, in this study, we tested the hypothesis that low vitamin A concentration at birth was associated with higher incidence rate of acute

respiratory infections, and with rates of admission for lower respiratory tract infections in the first five years of life.

Participants of this study were originally recruited for evaluation of prenatal exposure to food chain contaminants, results to be published separately.

Material and method

Study population and recruitment

The recruitment procedure for this study was previously described (Dallaire et al. 2003). In the current analysis, only participants from Nunavik were included. Briefly, between 1993-1997, pregnant women arriving for delivering at one of the two participating health centers were invited to participate. Umbilical cord blood was sampled and a post-partum interview was conducted few days after delivery. In the current study, the participants were children born to these mothers. Women with at-risk pregnancies who gave birth in a tertiary center were not recruited. Therefore, our study population had a bias towards healthier pregnancies.

A subgroup of mother-child pairs was also selected for a 5-year follow-up interview ($n = 88$). In this subgroup, adopted children were excluded because the consent form that allowed us to contact them was signed by the biological mother. Children who were younger or older than 5 years old at the time our staff visited their community were also excluded from that subgroup.

Medical chart review and infection incidence rate

We attempted to review the medical charts of all children included in our previous study for the first 5 years of age. We made a list of all the medical chart numbers available in our database and worked with the staff of the communities' health centers to locate the charts. Then, copies of the charts were sent to our research center to be reviewed by five 2nd- and 3rd-year trained medical students using a standardized questionnaire. For every diagnosis of infection noted in the charts, we recorded the date of diagnosis, whether antibiotics were prescribed, and whether the child was hospitalized. When the child was hospitalized, we recorded the main reason of hospitalization, whether concurrent illnesses were present, the

date of hospitalization, the length of stay, and whether the child was transferred to another hospital. For the present study, only acute respiratory infections were targeted. Four categories were created: upper respiratory tract infections (URTIs), lower respiratory tract infections (LRTIs), hospitalization for lower respiratory tract infections, and acute otitis media (AOM). AOM was analyzed separately from URTIs because AOM is a significant, well-recognized problem in Nunavik. The URTIs category included streptococcal pharyngitis and tonsillitis, acute upper respiratory tract infection not otherwise specified (NOS), acute rhinitis, head cold, nasopharyngitis, pharyngitis, coryza, sinusitis, tonsillitis, laryngitis, tracheitis, croup, and influenza. The LRTIs category included acute bronchitis and bronchiolites, acute lower respiratory infection NOS, chest infection NOS, laryngotracheobronchitis, tracheobronchitis, bacterial and viral pneumonia, bronchopneumonia, influenzal pneumonia, and pneumonitis. For ear infections, only acute otitis media were included. Otitis media with effusion, chronic otitis media and glue ears were excluded.

Two infectious episodes affecting the same anatomic site were considered separate if there was at least fifteen days between the two diagnosis and if it was not specified in the chart that the second episode was related to the first.

Determination of vitamin A in cord blood

Detailed analytic method was previously described (Dallaire et al. 2003). Briefly, blood samples were centrifuged, frozen at 80°C and then sent to the Institut National de Santé Publique du Québec (Québec City, Canada) for vitamin A (retinol) analysis. Retinol concentration was determined by reversed-phase high-pressure liquid chromatography.

Data collection on confounding factors

The selection of potential confounding variables was based on clinical knowledge and a literature review. Perinatal factors were documented using data from the medical charts review, and from the postpartum interview. These factors were, sex of the child, birth weight, reviewer of the medical chart, gestational age, and smoking during pregnancy. Data on crowding and socioeconomic status was gather through the 5-year follow-up interview.

Statistical analyses

Poisson regression was used to evaluate the associations between the vitamin A concentration and the infection incidence rates. The main dependant variables were the number of diagnosed episodes of infection during the first five years of life, and the main independent variable was vitamin A concentration in cord blood. Two regression models were constructed: one in which vitamin A was treated in categories [< 10µg/dl, 10-14µg/dl, 15-19µg/dl and ≥ 20µg/dl (reference)], and one in which it was treated in continuous. The continuous model yielded a single RR corresponding to the relative increase in rate for each 5 µg/dl increase in the concentration of vitamin A.

Adjustment for confounding factors was done using multiple regression (Poisson regression). Potential confounding factors were tested in the model one by one, but only those influencing the incidence rate ratios (RRs) by more than 5% were included in the final model. The variables initially excluded were retested one by one in the final model to ensure that their exclusion did not influence the results. The variables included in the final multivariate model were sex and birth weight (dichotomous, < 3000 g or ≥ 3000 g). Gestational age, smoking during pregnancy, contaminant exposure, and reviewer of the chart were excluded. Some potential confounding factors were available only for children included in the 5-year follow-up subgroup. These factors were breastfeeding duration, crowding, and socioeconomic status. None of these variables influenced the association in a significant manner and they were also excluded from the final model.

We used SPSS Data Entry Builder 2.0 for data entry (Chicago, Illinois, United States) and SAS 8.02 (Cary, NC, United States) for database management and statistical analyses. A *p*-value < 0.05 was considered significant.

Results

Participants

Three hundred seventy-five children from Nunavik were initially included in the first phase of this study (Dallaire et al. 2003). Of these 375 participants, it was impossible to get the chart of 31 (8.3%) children for various logistical reasons. Among the 344 available charts, 23 (6.7%) were incomplete, 13 (3.8%) families moved out of Nunavik during follow-up, 4

(1.2%) children died and 3 (0.9%) children were excluded because they suffered from a serious chronic disease. The final analysis included the 301 remaining children. Four additional children who were excluded from the first phase of this study because of missing values were included in this analysis. Table 11.1 shows the characteristics for all participants.

Table 11.1
Characteristics of participants

Characteristics	**Mean Value or percentage** (n = 305)
Children	
Sex (male)	47.7%
Mean gestational age	39.1 weeks
Preterm (< 37 weeks)	5.2%
Birth weight	3503 g
Length	51.5 cm
Mothers	
Age	23.8 years
Parity	2.1

Vitamin A concentrations

The mean vitamin A concentration in cord blood for all participants was 15.7 µg/dl ranging from 5.5 µg/dl to 30.8 µg/dl. There were 8.5% children with < 10 µg/dl, 38.7% children with 10-14 µg/dl, 35.7% children with 15-19 µg/dl, and 17.0% children with ≥ 20 µg/dl.

Infection incidence rates

A total of 4846 outpatient visits that lead to a diagnosis of acute respiratory infection before the age of five were identified. Incidence rates for AOM, URTIs, LRTIs and hospitalization for LRTIs are shown in table 11.2. Acute otitis media was the most frequently diagnosed infection, followed closely by upper respiratory tract infections. Hospitalizations were frequent as 17.5% of outpatient visits for LTRIs led to an admission.

Table 11.2
Incidence rate of acute respiratory infections

Infection and Hospitalization	**Incidence rate** (events per 1000 child-years)
Acute otitis media	1254 (1149 – 1359)
Upper respiratory tract infections	1244 (1142 – 1346)
Lower respiratory tract infections	628 (562 – 694)
Hospitalization for Lower respiratory tract infections	110 (85 – 134)

Vitamin A concentration and infections

Table 11.3 shows the association between acute respiratory infections and vitamin A concentration in cord blood. For AOM, compared to children in the ≥ 20 μg/dl group, children in vitamin A groups of < 10 μg/dl, 10-14 μg/dl, and 15-19 μg/dl had RRs of 1.63, 1.25, and 1.08, respectively. Statistical significance was reached for children with < 10 μg/dl ($p < 0.0001$), and for those with 10-14 μg/dl ($p < 0.05$). A statistically significant negative association ($p<0.0001$) was also observed in the continuous model (RRs = 0.88 for each 5 μg/dl increase of vitamin A concentration, $p < 0.0001$). Similar results were observed in the adjusted model.

Table 11.3
Incidence rate ratio of acute respiratory infections and hospitalizations for lower respiratory tract infections according to vitamin A concentration in cord blood

Vitamin A concentration in cord blood	Incidence rate ratio (95 % CI)			
	AOM	URTIs	LRTIs	Hospitalizations for LRTIs
Unadjusted model (*n* = 305)				
Continuous (for each 5 µg/dl increase)	0.88 (0.845 - 0.92)**	1.00 (0.95 - 1.04)	0.92 (0.87 - 0.99)*	0.81 (0.68 - 0.97)*
Categories (µg/dl)				
< 10	1.63 (1.37 - 1.94)**	1.00 (0.83 - 1.22)	1.17 (0.88 - 1.55)	1.43 (0.74 - 2.77)
10 – 14	1.25 (1.09 - 1.44)*	1.07 (0.93 - 1.22)	1.31 (1.07 - 1.60)*	1.66 (1.02 - 2.68)*
15 – 19	1.08 (0.94 - 1.24)	1.14 (0.99 - 1.30)	1.34 (1.10 - 1.64)*	1.18 (0.71 - 1.96)
≥ 20	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)
Adjusted model (*n* = 301)				
Continuous (for each 5 µg/dl increase)	0.87 (0.83 - 0.92)**	1.00 (0.95 - 1.05)	0.93 (0.87 - 1.01)	0.87 (0.73 - 1.05)
Categories (µg/dl)				
< 10	1.62 (1.35 - 1.95)**	1.01 (0.82 - 1.24)	1.12 (0.83 - 1.50)	1.14 (0.57 - 2.27)
10 – 14	1.25 (1.08 - 1.44)*	1.07 (0.93 - 1.23)	1.27 (1.03 - 1.56)*	1.43 (0.86 - 2.37)
15 – 19	1.06 (0.92 - 1.23)	1.16 (1.01 - 1.33)*	1.34 (1.09 - 1.64)*	1.09 (0.64 - 1.84)
≥ 20	1.00 (referent)	1.00 (referent)	1.00 (referent)	1.00 (referent)

AOM = acute otitis media, URTIs = upper respiratory tract infections, LRTIs = lower respiratory tract infections, CI = confidence interval.
* Statistically significant ($p < 0.05$).
** Statistically significant ($p < 0.0001$).

For URTIs, there was no significant association except for the 15-19 µg/dl group in the adjusted model (RR = 1.16). For LRTIs, compared to children in the ≥ 20 µg/dl group, children in vitamin A groups of < 10 µg/dl, 10-14 µg/dl, and 15-19 µg/dl had RRs of 1.17, 1.31, and 1.34, respectively. Statistical significance was reached for children with 10-14 µg/dl ($p < 0.05$), and for those with 15-19 µg/dl ($p < 0.05$). The dose-response relationship that was apparent for AOM in the categorical was not observed for LRTIs. Nevertheless, the continuous model yielded a statistically significant negative association (RRs = 0.92 for each 5 µg/dl increase of vitamin A concentration, $p < 0.05$). Results were similar in the adjusted model, but statistical significance was lost in the adjusted continuous model.

For hospitalization for LRTIs, children in lower vitamin A category all had RRs above 1.0, but statistical significance was reached only for children 10-14 µg/dl in the unadjusted model (RR = 1.66, $p < 0.05$). The continuous model showed a negative association (RRs = 0.81 for each 5 µg/dl increase of vitamin A concentration, $p < 0.05$), but the statistical significance was lost in the adjusted model.

Discussion

This study was undertaken in order to document the association between vitamin A concentration in cord blood and acute respiratory infections during the first five years of life in Inuit children. A statistically significant association between lower vitamin A concentration at birth and higher incidence rate was found for AOM and LRTIs, but not for URTIs and hospitalization for LRTIs. For AOM, the association showed a dose-response pattern.

Reports of placebo-controlled prospective studies on supplementation are controversial as some authors found that vitamin A supplementation decreased respiratory infections incidence rates (Pinnock et al. 1986, Sempertegui et al. 1999) while some did not (Barreto et al. 1994, Basu et al. 2003, Biswas et al. 1994, Kartasasmita et al. 1995, Venkatarao et al. 1996). In observational prospective studies, vitamin A deficiency was associated with rate of respiratory infections (Pandey & Chakraborty 1996, Sommer et al. 1984) but not with rate of AOM (Durand et al. 1997). In a cross-sectional, follow-up and interventional trial study performed in Thailand with a similar deficient population, Bloem et al. (1990) found a dose-response relationship between respiratory diseases incidence rates in children and mild vitamin A deficiency.

Our categorical model yielded an apparent dose-response relationship for AOM, but not for LRTIs and hospitalization. Nevertheless, for LRTIs, rate ratios for children in the 10-14 µg/dl and 15-19 µg/dl categories were significant. Furthermore, the continuous model for LRTIs was significant in the unadjusted model, and borderline significant in the adjusted model. These results suggest two possibilities. First, there could be an increased incidence of LRTIs in children with < 20 µg/dl, but we lacked statistical power to identify a clear dose-response pattern in the categorical model. Second, there could exist at cut-off vitamin A value below which the susceptibility to LRTIs is increased without further dose-response relationship. Further studies with a greater number of participants are needed to resolve this issue. In the previous phase of this study, we argued that the cut-off concentration for vitamin A deficiency in umbilical cord blood was 10 µg/dl (Dallaire et al. 2003). The results of the present study, however, show that although no specific clinical effect of vitamin A deficiency, such as night blindness, were apparent above 10 µg/dl, a possible

adverse health effect could be present for children below 20 µg/dl. However, because of the unique socioeconomic situation of the Inuit children, it would be hard to infer from our results that such an effect could be present in non-Inuit populations.

It has been discussed by some authors that the effect of a deficient level of vitamin A could better be observed on the severity of infectious episodes rather than on the incidence rate (Roy et al. 1997). Indeed, an association between vitamin A level and severity has been demonstrated in some studies (Basu et al. 2003, Julien et al. 1999), but not all (Kartasasmita et al. 1995). To assess the impact of deficient vitamin A level at birth on the severity of infections in our population, we examined the incidence of hospitalization for LRTIs. Unfortunately, we lacked statistical power and no significant association was found. However, associations were positive and the effect-size in both the categorical and the continuous models was greater when only LRTIs that led to an admission were considered, compared to the total rate of LRTIs. This suggests that not only the rate of LRTIs is increased in children with lower vitamin A levels, but that these episodes were also more severe. Further studies with a greater number of subjects are needed to clarify this issue.

In the present study, a medical chart review was used to evaluate incidence rates of infection. There is only one health center in each community included in this study and participants always go to that health center when they seek medical care. Copies of consultations done elsewhere are also routinely requested to complete local medical charts. We are therefore confident that we have reviewed the majority of outpatient visits. However, parent's decision of seeking medical attention is related to many cultural factors, which in turn could be associated with dietaries habits. A bias could therefore be introduced if the propensity to seek medical attention was associated with low or high vitamin A consumption. If this bias was present in our data, it is likely that it would be insignificant when severe symptoms were present, symptoms for which every parent would go to the clinic. It would also not be present for hospitalization, as the decision is not one of the parents. We cannot, however, exclude the possibility of a biased association for AOM or URTIs.

Nutrition status is an important factor that can modulate the immune system. Our preliminary analyses allowed us to find that socioeconomic status and crowding, two

factors that could be related to malnutrition, did not influence the association between vitamin A and infection rate. However, we found that vitamin A concentration was correlated with omega-3 fatty acids, which means that a greater maternal fish consumption – and most likely a more traditional diet – was associated with a greater vitamin A intake (results not shown). It also means that a lower vitamin A concentration could be associated with a diet composed of a greater proportion of imported food, a diet that is often less-nutritive that the traditional inuit diet (Blanchet et al. 2000). Therefore, we cannot exclude that part of the association between vitamin A and infection rate could be due to other nutriments deficiencies, or to a generally less healthy diet. We did however exclude the potential confounding effect of organochlorines exposure because the association found between prenatal organochlorines exposure and infections in this population was completely independent from the association between neonatal vitamin A and infections (data not shown).

This study underlines a possible link between low vitamin A concentration at birth and acute respiratory infections in Inuit children from Nunavik. Because children from this population are burdened by a high incidence of AOM and LRTIs compared to other North-American populations, the identification of a preventable risk factor such as vitamin A deficiency is of paramount importance. Together with the first phase of this study, these results indicate that a carefully planned supplementation program should be considered for both pregnant women and newborns of Nunavik.

Acknowledgment

We are grateful to the Nunavik population for their participation in this research. We thank Marie-Lise Mercier, Mélanie Gaudreault, Catherine Lalonde, Élisabeth Leblanc, and Valérie Marchand for medical charts review, and Patsy Tulugak and Mary Nulukie for help with charts retrieval and copying. We are indebted to Daria Pereg for her valuable inputs during the preparation of this manuscript.