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Association entre les métaux, les amalgames dentaires, les phénols et les désordres hypertensifs de la grossesse

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Résumé

Très peu demeure connu sur le rôle de l'exposition à différents métaux ou autres agents chimiques dans le développement des désordres hypertensifs de la grossesse (DHG). Cette thèse porte sur l'évaluation de l'association potentielle entre les niveaux des métaux (l'arsenic, le plomb, le cadmium, le mercure ou le manganèse), du bisphénol A (BPA), du triclosan (TCS) ou l'exposition aux amalgames dentaires et les DHG chez les femmes canadiennes.

Nous avons conçu et mené 3 analyses, toutes basées sur les données de l'Étude mère-enfant sur les composés chimiques de l'environnement (MIREC), une cohorte de 2001 femmes enceintes recrutées dans 10 villes canadiennes entre 2008 et 2011 et suivies avec leur enfant dans le but d'étudier la relation entre des expositions à des contaminants chimiques et la santé des mères et de leurs enfants. Le statut des amalgames dentaires (présence ou remplacement) et les concentrations sanguines des métaux ont été évalués au premier et au troisième trimestre tandis que les concentrations urinaires de l'arsenic, du BPA et du TCS ont été mesurées au premier trimestre de grossesse. La tension artérielle (TA) a été mesurée à chaque trimestre de la grossesse. Les rapports de cotes ajustés (aOR) et les intervalles de confiance (IC) à 95% ont été estimés par la régression logistique et multinomiale pour déterminer les associations entre les métaux, les amalgames dentaires, le BPA ou le TCS et l'hypertension gestationnelle globale (HGG) et ses deux sous-catégories : l'hypertension gestationnelle et la prééclampsie. Des équations d'estimation généralisées linéaire et logistique et des régressions linéaires ont aussi été effectuées pour analyser les associations entre ces contaminants chimiques et la TA ou HGG.

Un niveau sanguin élevé de manganèse au premier trimestre (> 9.89 µg/l) était associé à une diminution du risque de HGG (aOR= 0.68; 95% IC : 0.46, 0.99) et de la prééclampsie (aOR= 0.48; 95% IC : 0.23, 0.98). Avec la stratification, cette association n'a été observée que chez les mères ayant accouché de bébés mâles (aOR= 0.41; 95% IC : 0.24, 0.70) contrairement aux mères des bébés femelles (aOR= 1.00; 95% IC : 0.92, 1.08). Par ailleurs, lorsque les mesures concurrentes sont effectuées entre le manganèse et la TA au premier et au troisième trimestre de la grossesse, on a observé une association positive (beta= 1.52 mmHg (0.80, 2.24)). Un niveau sanguin élevé de mercure au troisième trimestre (> 0.82 µg/l) était lié à une diminution du risque de HGG (aOR= 0.62; 95% IC: 0.39, 0.98). L'arsenic sanguin (en variable continue) mesuré au troisième trimestre, était associé à un risque plus élevé de la prééclampsie (aOR= 1.16; 95% IC : 1.03, 1.30). Aucune association significative n'a été observée pour le plomb, le cadmium, l'arsenic urinaire, le statut d'amalgame dentaire, le BPA ou le TCS.

Dans l'ensemble, la thèse a permis de montrer une association entre le manganèse, le mercure ou l'arsenic et les DHG. Par contre, elle n'a pas montré de lien entre le plomb, le cadmium, l'arsenic urinaire, les amalgames dentaires, le BPA ou le TCS et les DHG.

Mots-clés : métaux, BPA, TCS, amalgames dentaires, restaurations dentaires, hypertension, hypertension gestationnelle, prééclampsie, grossesse, contamination environnementale.

English Summary

Very little remains known about the role of exposure to different metals or other chemical agents in the development of hypertensive disorders of pregnancy (HDP). In this thesis, we aimed to assess the potential association between the levels of metals (arsenic, lead, cadmium, mercury or manganese), bisphenol A (BPA), triclosan (TCS) or exposure to dental amalgams and HDP among Canadian women.

We designed and conducted 3 analyses, all based on the databank of the Maternal-Infant Research on Environmental Chemicals (MIREC) Study, a cohort of 2001 pregnant women recruited in 10 Canadian cities between 2008 and 2011 and followed up with their children to study the relationship between chemical contaminant exposures and maternal and child health. The status of dental amalgams (presence or replacement) and blood concentrations of metals were evaluated in the first and third trimesters, while urinary concentrations of metals, BPA and triclosan TCS were measured in the first trimester. Blood pressure (BP) was measured in each trimester of pregnancy. Adjusted odds ratios (aOR) and 95% confidence intervals (CI) were estimated by logistic and multinomial regression to measure the associations between metals, dental amalgams, BPA or TCS and HDP (gestational hypertension overall and its two subcategories: gestational hypertension and preeclampsia). Linear and logistic generalized estimation equations and linear regression were also performed to measure the associations with BP or gestational hypertension overall.

The highest category of first trimester blood manganese level (> 9.89 µg /l) was associated with a reduced risk of gestational hypertension overall (aOR= 0.68, 95% CI: 0.46, 0.99) and preeclampsia (aOR= 0.48, 95% CI: 0.23, 0.98). With the stratified analysis, this

association was observed only in mothers who gave birth to male babies (aOR= 0.41; 95% IC: 0.24, 0.70) as opposed to mothers of female babies (aOR= 1.00; 95% IC: 0.92, 1.08). However, when manganese and BP were measured concurrently during the same trimester of pregnancy, a positive association was observed (beta= 1.52 mmHg (0.80, 2.24)). A higher blood level of mercury in the third trimester (> 0.82 µg/l) was associated with a lower risk of gestational hypertension overall (aOR for the third tertile = 0.62; 95% CI: 0.39, 0.98). With respect to blood arsenic (in continuous variable) measured in the third trimester, it was associated with a high risk of preeclampsia (aOR= 1.16; 95% IC: 1.03, 1.30). No significant association was observed for lead, cadmium, urinary arsenic, dental amalgams status, BPA or TCS.

Overall, the thesis showed an association between manganese, mercury or arsenic and HDP. However, it did not show a link between lead, cadmium, urinary arsenic, and dental amalgams, BPA or TCS and HDP.

Keywords: metals, BPA, TCS, dental amalgams, dental restorations, hypertension, gestational hypertension, preeclampsia, pregnancy, environmental contamination.

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ABPM	Ambulatory blood pressure monitoring
ACOG	American college of obstetricians and gynaecologists
ASPC	Agence de Santé Publique du Canada
ARNm	Acide ribonucléique messager
CDC	Centers for Disease Control and Prevention in the U.S.A.
CIHR	Canadian Institutes of Health Research
CI	Confidence interval
IC	Intervalle de Confiance
DMA	Acide diméthylarsinique
ECMS	Enquête canadienne sur les mesures de la santé
FDA	Food and Drugs Administration
FRQ-S	Fonds de la recherche du Québec en santé
GC-MS/MS	Gas Chromatography coupled with tandem Mass Spectrometry
GEE	Generalized estimation equation
GerES	German Environmental Survey III et IV
GRP78	Glucose-regulated protein
HBPM	Home blood pressure monitoring
HPLC	High performance liquid chromatography
ICP-MS	Inductively coupled plasma mass spectrometry
INSPQ	Institut national de santé publique du Québec
IL-6	Interleukin 6
IL-8	Interleukin 8
INRS	Institut National de Recherche et de Sécurité
KNHES	Korean National Health Examination Survey
LC-MS/MS	Liquid Chromatography coupled with tandem Mass Spectrometry
MIREC	Maternal-Infant Research on Environmental Chemicals study
MMA	Acide monométhylarsonique
MPM	métalloprotéinase matricielle
NHANES	National Health and Nutrition Examination Survey
NHBPEP	National High Blood Pressure Education Program
OMS	Organisation Mondiale de la Santé
OR	Odds ratio
P	P-values
PlGF	Placenta Growth Factor
PVC	Polyvinyl chloride
QTNPR	Quebec Training Network in Perinatal Research
SD	Standard deviation
SOGC	Société des Obstétriciens et Gynécologues du Canada
sEng	soluble Endoglin
sFlt-1	soluble Factor fms-like tyrosine Kinase-1
TNHES	Thai National Health Examination Survey
TNF-alpha	Tumor Necrosis Factor-Alpha

VEGF	Vascular endothelial growth factor
WHO	World Health Organization

Liste des abréviations

BP	Blood pressure
DHG	Désordres hypertensifs de la grossesse
GH	Gestational Hypertension
GM	Geomeric mean
MG	Moyennes géométriques
HDP	Hypertensive disorders of pregnancy
HGG	Hypertension gestationnelle globale
IMC	Indice de masse corporelle
IQR	Interquartile range
LDD	Limite de détection
MAINC	Ministère des affaires Indiennes et du Nord Canada
SESST	Service de l'environnement et de la santé et sécurité au travail
TA	Tension artérielle
TAS	Tension artérielle systolique
TAD	Tension artérielle diastolique

Dédicace

Je dédie ce travail à ma famille qui n'a jamais perdu la foi en moi.

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Chapitre 1 - Introduction

Les désordres hypertensifs de la grossesse touchent environ 3.6% à 9.1% des femmes enceintes dans les pays développés (Roberts et al. 2011). Ils représentent l'une des causes majeures de mortalité (Berhan and Endeshaw 2015; Lo et al. 2013; Moodley 2004; Nakimuli et al. 2016; Riaz et al. 2011) et de morbidité maternelles (Lykke et al. 2009; Nakimuli et al. 2016; Riaz et al. 2011). Selon Khan et al. (2006), les désordres hypertensifs de la grossesse sont associés à 25.7% des décès maternels en Amérique latine et dans les Caraïbes, 16.1% dans les pays développés et 9.1% en Afrique et en Asie. La prééclampsie et l'éclampsie représentent 2 à 8% des grossesses et sont responsables de 10 à 15% des décès maternels (Duley 2009). Au Canada, le taux de mortalité maternelle par hypertension reliée à la grossesse, l'accouchement et la puerpéralité est de 1.4 par 100 000 accouchements avec un intervalle de confiance (IC) à 95% de 1.0 à 1.9 (ASPC 2013).

L'étiologie des désordres hypertensifs de la grossesse reste encore inconnue (Clivaz et al. 2007; Feihl et al. 2009) ou incertaine (Robillard et al. 2011). Cependant plusieurs facteurs évoqués dans leur physiopathologie convergent vers le stress oxydatif (Agarwal et al. 2012; Álvarez and Acosta 2000; Cunningham et al. 2010; Lefèvre et al. 1997; Reslan and Khalil 2010; Vaziri and Sica 2004). Parmi les causes possibles du stress oxydatif, on retrouve les expositions environnementales dont l'exposition aux métaux (Favier 2003), au bisphénol A (BPA) (Asimakopoulos et al. 2015; Rezg et al. 2014) et au triclosan (TCS) (Canesi et al. 2007). Il est donc plausible de penser que l'exposition des femmes enceintes à ces agents chimiques soit un des facteurs de risque dans le développement de l'hypertension, et ceci à travers un processus affectant le stress oxydatif (Houston 2007; Kennedy et al. 2012; Vaziri and Sica 2004). Par ailleurs, certaines études ont montré une association entre le plomb (Pb) (Yazbeck et al. 2009), le

manganèse (Mn) (Vigeh et al. 2013), le cadmium (Cd) (Kosanovic and Jokanovic 2007) ou le mercure (Hg) (Pan et al. 2007) et le risque d'hypertension gestationnelle. Une étude a trouvé une association entre l'arsenic (As) mesuré dans l'urine et la tension artérielle (TA) durant la grossesse (Farzan et al. 2015). Des associations ont été aussi montrées entre le Pb (Ikechukwu et al. 2012; Jameil 2014), le Cd (Laine et al. 2015; Pollack et al. 2014) et le Mn (Maduray et al. 2017) et la prééclampsie. Pour le BPA, la possibilité d'une association avec la prééclampsie a été soulevée (Cantonwine et al. 2016; Leclerc et al. 2014).

L'exposition aux métaux (As, Pb, Cd, Hg) demeure un problème de santé publique (Tchounwou et al. 2012). Au cours des 50 dernières années, l'exposition aux métaux a augmenté dans le monde en raison de l'essor de l'industrialisation (Huss 2011). Par contre, les niveaux des métaux demeurent faibles dans certains pays industrialisés (Wong and Lye 2008) par rapport à certains pays moins industrialisés (Ghazali et al. 2012; Orisakwe 2014). La toxicité des métaux est favorisée d'une part par leur demi-vie biologique qui peut aller jusqu'à 15 ou 30 ans chez l'humain (INRS 1997, 2013; Järup 2003; WHO 1995) et d'autre part par leur rémanence dans l'environnement (Huss 2011; Wong and Lye 2008). Malgré la baisse de l'utilisation industrielle des métaux tels que le Pb, les métaux demeurent problématiques pour la santé publique au Canada (Wong and Lye 2008). En effet, l'enquête canadienne sur les mesures de la santé (ECMS) a fourni le profil d'exposition aux métaux (Pb, Cd et Hg) pour la population générale dont près de 1% des individus présentent des niveaux au-dessus des normes (Wong and Lye 2008). Avant l'étude de cohorte MIREC (Maternal-Infant Research on Environmental Chemicals), il n'existait pas de données nationales pan-canadiennes des niveaux d'exposition aux métaux pour les femmes enceintes. Le projet MIREC est la première étude canadienne qui dresse le profil d'exposition aux contaminants chimiques environnementaux des femmes enceintes et

leurs enfants et étudie leurs effets potentiels sur la santé des mères et de leurs enfants. Plusieurs types ou sources d'exposition à différents métaux ou encore d'exposition à des agents chimiques ont été mesurés chez des femmes recrutées dans différents centres obstétriques canadiens durant le premier trimestre de grossesse. Les chercheurs de MIREC ont déjà publié une analyse de la distribution des niveaux des métaux dans la population de l'étude (Arbuckle et al. 2016). Ce projet de doctorat représente un volet de l'étude MIREC et a pour objectif d'examiner l'association entre 1) l'exposition à différents métaux (As, Pb, Cd, Hg ou Mn), 2) la présence ou le remplacement des amalgames dentaires (source d'exposition au Hg principalement) ou 3) l'exposition au BPA ou au TCS et les désordres hypertensifs de la grossesse.

Cette thèse par articles présente, après cette introduction, les chapitres 2 et 3 qui font respectivement état de la recension des écrits et des objectifs et des hypothèses de la thèse. Le chapitre 4 présente la méthodologie. Les chapitres 5, 6 et 7 présentent les 3 articles de la thèse qui se termine par les chapitres 8 et 9 qui présentent respectivement la discussion et la conclusion.

Chapitre 2 - Recension des écrits

2.1 Les désordres hypertensifs de la grossesse

Selon la Société d'Obstétriciens et de Gynécologues du Canada (SOGC), les désordres hypertensifs de la grossesse sont définis par la présence d'une tension artérielle systolique (TAS) ≥140 et/ou de la tension artérielle diastolique (TAD) ≥90 mmHg. Les mesures sont basées sur la moyenne d'au moins deux prises de la TA à au moins 15 min d'intervalle (Magee et al. 2014). Une TAS ≥160 et/ou TAD ≥110 définit la forme sévère de l'hypertension artérielle (Magee et al. 2014).

2.1.1 La classification des désordres hypertensifs de la grossesse

Selon la classification de la SOGC (Magee et al. 2014), les désordres hypertensifs de la grossesse peuvent être regroupés en quatre catégories : l'hypertension préexistante (chronique), l'hypertension gestationnelle, la prééclampsie et les autres effets hypertensifs (tableau 1). Chaque catégorie présente une définition et des caractéristiques particulières qui sont présentées dans le tableau 1.

Tableau 1. Définition et caractéristiques de chaque catégorie des désordres hypertensifs de la grossesse selon la SOGC.

Désordres hypertensifs de la grossesse		**Définition et caractéristiques**
Hypertension pré-existante (chronique)		Une tension artérielle (TA) ≥ 140/90 mmHg avant la grossesse ou la 20eme semaine de gestation.
	Avec comorbidité	Inclut une ou des comorbidités (par exemple, le diabète pré-gestationnel de type I ou II ou une maladie rénale)
	Prééclampsie surajoutée à l'hypertension chronique	● hypertension résistante, ou ● nouvelle apparition de la protéinurie (≥ 0.3 g/24-h ou ≥ 30 mg/mmol de créatinine urinaire, ≥ 1+ à la bandelette urinaire) ou qui s'aggravent, ou ● une ou plusieurs des conditions défavorables, ou ● un ou des complications plus graves Prééclampsie sévère est définie comme la prééclampsie avec une ou plusieurs des complications graves.
Hypertension gestationnelle		Définie comme l'hypertension qui se développe pour la première fois à l'âge gestationnel ≥ 20 semaines.
	Avec d'autres comorbidités	Les comorbidités (par exemple, le diabète pré-gestationnel type I ou II ou une maladie rénale) justifient un contrôle plus strict BP en dehors de la grossesse en raison de leur association avec le risque cardiovasculaire accru.
	Avec la prééclampsie	Évidences de la prééclampsie peuvent apparaître que plusieurs semaines après le début de l'hypertension gestationnelle. La prééclampsie est définie comme l'hypertension gestationnelle avec un ou plusieurs des éléments suivants: ● nouvelle protéinurie, ou ● une ou plusieurs des conditions défavorables, ou ● une ou des complications plus graves. Prééclampsie sévère est définie comme la prééclampsie avec une ou plusieurs des complications graves.
Prééclampsie		La prééclampsie peut survenir de novo. EIle est définie comme une hypertension gestationnelle avec un ou plusieurs des éléments suivants : ● nouvelle protéinurie, ou ● une ou plusieurs des conditions défavorables, ou ● une ou des complications plus graves. Prééclampsie sévère est définie comme la prééclampsie avec une ou plusieurs des complications graves.
Autres effets hypertensifs		Peuvent se produire chez les femmes dont TA est élevée à <20 + 0 ou ≥ 20 + 0 semaines et qui sont soupçonnées d'avoir hypertension préexistante ou gestationnelle/ prééclampsie, respectivement.
	Effet hypertenseur transitoire	TA élevée peut être due à des stimuli environnementaux, par exemple, la douleur de l'accouchement.
	Effet hypertenseur de la blouse blanche	TA qui est élevée dans le bureau (TAS ≥ 140 mmHg ou diastolique ≥ 90 mmHg), mais constamment normale en dehors du bureau (<135/85 mmHg) par ABPM (surveillance ambulatoire de la TA) ou HBPM (surveillance de la TA à la

		maison)
	Effet hypertenseur masqué	Défini comme TA qui est toujours normale lors des consultations (TAS <140 mmHg ou diastolique <90 mmHg), mais élevée en dehors des consultations (≥ 135/85 mmHg) ABPM ou HBPM répétée.

Inspiré de Magee LA, Pels A, Helewa M, Rey E, Von Dadelszen P. 2014. Diagnosis, Evaluation, and Management of the Hypertensive Disorders of Pregnancy: Executive Summary. J Obstet Gynaecol Can 36:416–438.

Il existe d'autres classifications comme celle du groupe de travail du NHBPEP (National High Blood Pressure Education Program) de 2000 (Cunningham et al. 2010; Lenfant 2001) qui classifie les désordres hypertensifs de la grossesse selon les quatre groupes suivants : l'hypertension gestationnelle, l'hypertension chronique, la prééclampsie/éclampsie et la prééclampsie surajoutée à l'hypertension chronique. Les définitions spécifiques de cette classification sont présentées en annexe 1.

La définition des désordres hypertensifs de grossesse utilisée dans cette thèse repose sur la classification de la SOGC et inclut l'hypertension gestationnelle globale, l'hypertension gestationnelle et la prééclampsie (tableau 1). L'hypertension gestationnelle globale se réfère à l'hypertension qui apparaît à partir des vingt semaines de la grossesse avec ou sans la prééclampsie. L'hypertension gestationnelle sans protéinurie ou complications maternelles et la prééclampsie sont traitées comme les sous-catégories mutuellement exclusives de l'hypertension gestationnelle globale dans cette thèse. L'hypertension chronique n'a pas été considérée dans ce travail car seuls les désordres hypertensifs en lien exclusivement avec la grossesse sont l'objet de cette thèse de doctorat.

2.1.2 Les complications des désordres hypertensifs de la grossesse

Les différentes catégories des désordres hypertensifs de la grossesse peuvent présenter les complications suivantes (Leeman et al. 2016) :

- L'hypertension chronique peut être associée à la prééclampsie, au retard de croissance intra-utérin et à l'hématome rétroplacentaire.

- L'hypertension gestationnelle peut progresser vers la prééclampsie.

- La prééclampsie : peut conduire à l'éclampsie, l'œdème pulmonaire, l'oligurie, l'insuffisance rénale, des manifestations hépatiques (élévation des transaminases, douleur, hémorragie subcapsulaire et rupture capsulaire avec risque d'hémorragie intrabdominale), HELLP (hémolyse, enzymes hépatiques élevées, faible taux de plaquettes), le syndrome et la coagulation intravasculaire disséminée et des complications obstétricales (retard de croissance intra-utérin, l'hématome rétroplacentaire et la mort fœtale).

2.1.3 La physiopathologie des désordres hypertensifs de la grossesse

L'étiologie des désordres hypertensifs de la grossesse est incertaine (Rotaille et al. 2011) ou inconnue (Clivaz et al. 2007; Feihl et al. 2009). Cependant, dans leur physiopathologie sont invoqués les facteurs placentaires, immunologiques, génétiques, mécaniques (par compression des gros vaisseaux et vaisseaux utérins) et des lésions vasculaires préalables à la grossesse (Álvarez and Acosta 2000; Lefèvre et al. 1997; Grundmann et al. 2012; Reslan and Khalil 2010). Les facteurs placentaires sont considérés comme les plus importants (Agarwal et al. 2012; Álvarez and Acosta 2000; Reslan and Khalil 2010). Un cadre conceptuel résumant les mécanismes physiopathologiques sous-adjents à l'association entre les expositions à l'étude et les désordres hypertensifs de la grossesse est representé à la figure 1.

- **Les facteurs placentaires, le stress oxydatif et la métalloprotéinase matricielle (MPM)**

Les facteurs placentaires de la prééclampsie incluent le pauvre remodelage des artères spirales (8-

18 semaines de gestation) qui conduit aux lésions d'ischémie - reperfusion et au stress oxydatif des villosités choriales (Redman et al. 2014). Cette ischémie/reperfusion placentaire pourrait entraîner une production élevée des facteurs anti-angiogéniques (le facteur fms-like tyrosine Kinase-1 Soluble (sFlt-1) et l'endogline soluble (sEng)) et une diminution de la production des facteurs pro-angiogéniques (le facteur de croissance endothélial vasculaire (VEGF) et le facteur de croissance placentaire (PlGF)). De plus, il se produirait une libération des cytokines inflammatoires (l'interleukine 6 (IL-6), le facteur de nécrose tumorale alpha (TNF-alpha) et l'interleukine 8 (IL-8)), des espèces réactives d'oxygène (stress oxydatif), des facteurs inductibles par l'hypoxie et des anticorps contre les récepteurs vasculaires de l'angiotensine II (Agarwal et al. 2012; Álvarez and Acosta 2000; Athanassiades and Lala 1998; Clivaz et al. 2007; De Oliviera et al. 2010; Karumanchi and Levine 2010; Reslan et Khalil 2010; Vaziri et Sica 2004). Ces facteurs biologiquement actifs pourraient contribuer au développement de la dysfonction des cellules endothéliales maternelles (l'endothéliose vasculaire) qui entraînerait une production élevée des facteurs vasoconstricteurs (l'endothéline-1, l'angiotensine II, thromboxane A2) et une production réduite des facteurs vasodilatateurs (la prostacycline, l'oxyde nitrique et le facteur hyperpolarisant). Ces changements conduiraient à une augmentation de la résistance vasculaire et à l'apparition des désordres hypertensifs de la grossesse. L'endothéliose glomérulaire produirait la protéinurie (Agarwal et al. 2012; Álvarez and Acosta 2000; Athanassiades and Lala 1998; De Oliviera et al. 2010; Feihl et al. 2009; Karumanchi and Levine 2010; Lefèvre et al. 1997; Reslan and Khalil 2010; Vaziri and Sica 2004).

Le stress oxydatif est l'une des pistes possibles pouvant mener à une dysfonction des cellules endothéliales (Schulz et al. 2011). Il est défini comme étant un déséquilibre entre une

surproduction des marqueurs prooxydants comme les aldéhydes (dialdéhyde malonique) et les dienes conjugués et une diminution des antioxydants comme les vitamines E et C, le bêta-carotène et les enzymes antioxydantes (la glutathion peroxydase et la superoxyde dismutase) (Favier 2003; Lefèvre et al. 1997). Il pourrait être causé par des ischémies/reperfusion suivant des thromboses, les intoxications aux métaux et aux phénols (BPA et TCS), les anomalies génétiques limitant la production d'antioxydant et la carence nutritionnelle en antioxydants tels que les vitamines D, C et E, l'acide folique, le beta-carotène ou les oligo-éléments (Asimakopoulos et al. 2015; Canesi et al. 2007; Favier 2003; Gitto et al. 2002; Grundmann et al. 2012; Kim et al. 2013; Lefèvre et al. 1997; Reslan and Khalil 2010; Rezg et al. 2014).

Il semble que l'exposition au Pb (Engstrom et al. 2010; Gurer-Orhan et al. 2004), au Hg (Qian et al. 2004), au Cd (Angeli et al. 2013; Engstrom et al. 2010; Xu et al. 2003), à l'As (Ellinsworth 2015; Engstrom et al. 2010; Liu et al. 2003), au Mn (Cordova et al. 2013), au BPA (Asimakopoulos et al. 2015; Rezg et al. 2014) et au TCS (Canesi et al. 2007) pourrait amener le développement du stress oxydatif. Ce phénomène pourrait survenir en raison de la peroxydation lipidique (Mukherjee et al. 2007), l'inhibition de l'activité du superoxyde dismutase de Mn (Chtourou et al. 2011), la production accrue d'espèces oxygénées réactives telles que le peroxyde d'hydrogène, le superoxyde ou les radicaux hydroxyles (Cordova et al. 2013; Jomova et al. 2011) et l'altération dose-dépendante des niveaux d'ARNm de GRP78 (protéine régulée par le glucose: fournit une protection aux cellules soumises à un stress oxydatif) (Qian et al. 2001). Les espèces réactives de l'oxygène, qui conduisent au stress oxydatif peuvent altérer directement la fonction vasculaire ou causer des changements dans le tonus vasculaire (Schulz et al. 2011) à travers le déficit de l'oxyde nitrique (un puissant vasodilatateur) suite à son inactivation (Vaziri and Sica

2004). Ce mécanisme pourrait conduire à une augmentation de la résistance vasculaire périphérique pouvant aboutir à une élévation de la TA (Kennedy et al. 2012). De plus, les métaux, comme le Pb, pourraient augmenter la production de l'endothéline (substance vasoconstrictrice produite principalement par les cellules endothéliales) par un effet toxique direct sur les cellules endothéliales (Vaziri 2008; Vaziri and Sica 2004).

Une autre piste pouvant conduire à la dysfonction placentaire concerne la MPM qui est importante dans le remodelage vasculaire et utérin (Chen and Khalil 2017). Une expression anormale de MPMs, des cytokines et des intégrines utéroplacentaires pourrait conduire à une diminution de la tolérance immunitaire maternelle, à l'apoptose des cellules trophoblastiques invasives, à un remodelage inadéquat des artères spirales et une réduction de la pression de perfusion utérine. Cette dernière pourrait provoquer une dysfonction vasculaire généralisée, une augmentation de la vasoconstriction et l'hypertension durant la grossesse (Chen and Khalil 2017) à travers les mécanismes déjà mentionnés plus haut pour l'ischémie placentaire. Il existerait un lien entre les MPMs et les métaux (Au et al. 2016). Le Hg pourrait activer MPM-2 et MPM-9 (Jacob-Ferreira et al. 2009) et augmenter les niveaux de MPM-7 (Au et al. 2016). Le Cd inhiberait les activités de MPM-2 et MPM-9 (Lacorte et al. 2015). L'As a une relation positive et dose-dépendante avec MPM-2 et MPM-9 (Islam et al. 2015). Le Pb pourrait diminuer les niveaux de MPM-2 et accroître celui de MPM-9 (Au et al. 2016). Finalement le Mn induirait l'activité de MPM-2 (Zhang et al. 2002).

En somme, le stress oxydatif ou la MPM, influencé par des expositions aux métaux ou agents chimiques pourrait mener à une dysfonction des cellules endothéliales et placentaires et éventuellement conduire au développement des désordres hypertensifs de grossesse.

a- Les effets des nutriments

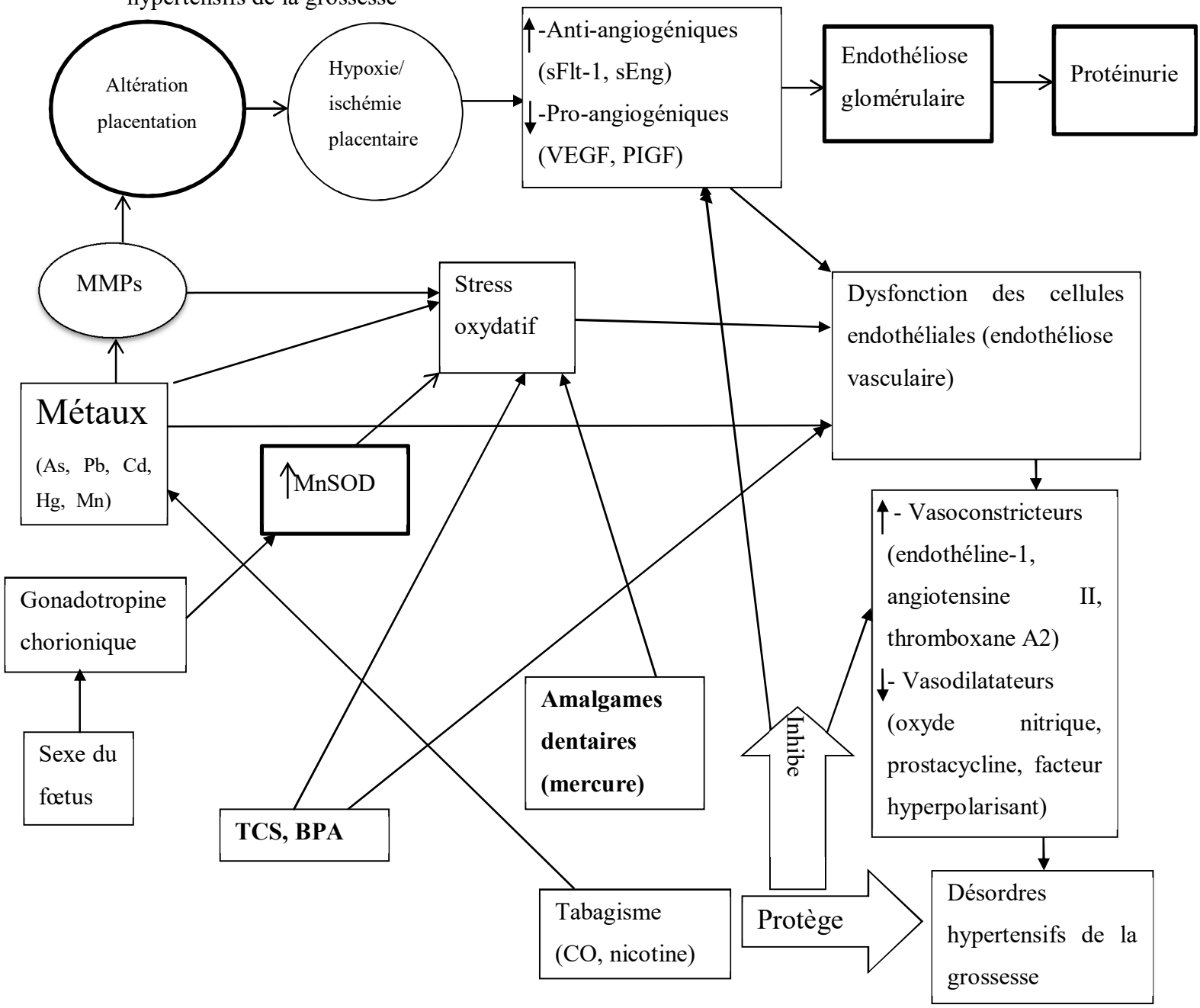
Calcium : Un régime alimentaire non équilibré peut entraîner des carences nutritionnelles de certains nutriments tel que le calcium, qui par le biais de différents mécanismes peut influencer les niveaux des métaux circulants comme la plombémie chez la mère (Johnson 2001; Motawei et al. 2013). Durant la grossesse, l'absorption maternelle du calcium au niveau intestinal s'accroît pour satisfaire les besoins de la croissance fœtale (Johnson 2001). Quand les apports en calcium par l'entremise de l'alimentation sont suffisants, son absorption intestinale accrue répond aux besoins de croissance du fœtus. Par contre si cet apport est insuffisant, le squelette de la mère devient alors une source importante de calcium pour le foetus (Johnson 2001). Ce processus de déminéralisation osseuse maternelle va également libérer dans la circulation maternelle le Pb bioaccumulé dans le compartiment osseux. Ce phénomène peut contribuer à augmenter la plombémie maternelle (Ettinger et al. 2006; Johnson 2001). De ce fait, les conséquences d'une supplémentation en calcium de >2000 mg/jour durant la grossesse incluent une diminution de l'absorption intestinale maternelle du Pb et la prévention de la résorption osseuse (Johnson 2001).

Autres nutriments : L'acide folique (Kim et al. 2013), le sélénium et les acides gras contenant de l'oméga-3 sont des antioxydants qui pourraient jouer un rôle actif dans la défense contre la toxicité maternelle des métaux tels que le Hg et le Cd (Houston 2007, 2011; Kantola et al. 2004). Par ailleurs, chez les animaux, certains auteurs ont montré que les vitamines C et E pouvaient avoir un effet réducteur sur le stress oxydatif (provoqué par le Cd) grâce à leurs propriétés antioxydantes (Beytut et al. 2003; Sen et al. 2004).

b- Les effets du tabagisme maternel

Le tabagisme maternel est une source de métaux (l'As, le Cd, le Pb, le Mn, le Hg, par exemple), de monoxyde de carbone et de nicotine (Chiba and Masironi 1992; FDA 2012; Vardavas et. al. 2011). Cependant, les études mentionnent une relation inverse entre le tabagisme maternel et le risque de l'hypertension gestationnelle (Marcoux et al 1989) et de prééclampsie (Marcoux et al 1989; Wikström et al. 2010). Pour expliquer cela, certains auteurs évoquent l'effet inhibiteur de la nicotine sur la production de thromboxane A_2 (Marcoux et al 1989). D'autres (Jeyabalan et al. 2008; Jeyabalan et al. 2010; Karumanchi and Levine 2010; Penney and Howley 1991; Wikström et al. 2010) considèrent les effets inhibiteurs du monoxyde de carbone sur la production placentaire des facteurs anti-angiogéniques. En plus, le tabagisme maternel est associé à des niveaux sériques élevés des facteurs pro-angiogéniques mais il est incertain si cela est en lien direct avec l'effet du monoxyde de carbone (Jeyabalan et al. 2008; Jeyabalan et al. 2010; Karumanchi and Levine 2010).

Figure 1. Le cadre conceptuel des mécanismes physiopathologiques sous-jacents aux désordres hypertensifs de la grossesse



Ce schéma est inspiré de la physiopathologie des désordres hypertensifs de la grossesse dans la littérature

2.2 Les métaux (le Pb, le Cd, le Hg, l'As et le Mn)

2.2.1 Les caractéristiques des métaux

Dans la littérature, les termes "métaux lourds" (Miquel 2001; Santé Canada 2014) ou "éléments traces métalliques" (Giroux et al. 2008) sont utilisés pour désigner les métaux. Selon Miquel (2001), "on appelle en général métaux lourds les éléments métalliques naturels, métaux ou dans certains cas métalloïdes caractérisés par une masse volumique élevée, supérieure à 5g/cm^3". Contrairement au Mn, l'As, le Pb, le Cd et le Hg n'ont aucune fonction physiologique connue (Jaishankar et al. 2014). Leur toxicité dépend de la dose, de la route d'exposition, de l'espèce chimique, de l'âge, du sexe, des facteurs génétiques, et du statut nutritionel de la personne exposée (Tchounwou et al. 2012). Les caractéristiques de chaque métal sont présentées dans le tableau 2.

Tableau 2 **:** caractéristiques des métaux

métaux	**caractéristiques**
Mn	- métal essentiel pour de nombreux processus physiologiques (Avila et al. 2013; Wood 2009) - joue un rôle de premier plan dans la fonction des enzymes, notamment le pyruvate carboxylase, la glutamine synthétase et le superoxyde dismutase (Avila et al. 2013; Wood 2009). Le pyruvate carboxylase est important dans le métabolisme du glucose, la glutamine synthétase intervient dans le métabolisme des astrocytes (Avila et al. 2013) et la superoxyde dismutase dans la défense contre les dommages causés par les radicaux libres (Candas et Li 2014). - sa toxicité peut également résulter d'une exposition élevée (manganisme (Avila et al. 2013), hypertension gestationnelle (Vigeh et al. 2013)) ainsi qu'une faible exposition (épilepsie, ostéoporose (Avila et al. 2013), prééclampsie (Sarwar et al. 2013)). - demi-vie : 4-39 jours pour le corps entier (Santé Canada 2016).
As	- métalloïde toxique (Järup 2003). - forme inorganique : As trivalent ou arsénite (As (III)) et As pentavalent ou arséniate (As (V)) qui sont méthylés en acide diméthylarsinique (DMA) et acide monométhylarsonique (MMA) (Jomova et al. 2011). - forme organique naturelle : l'arsénobétaïne, l'arsénocholine et l'As dérivé de sucres (Jomova et al. 2011). - excrété par l'urine principalement sous la forme méthylée et DMA est le plus abondant métabolite présent dans l'urine après exposition à l'As inorganique (60-80%) (Drobna et al. 2009). - DMA et MMA sont considérés moins toxiques (Jomova et al. 2011) ainsi que la forme organique naturelle (Jomova et al. 2011; Suzuki et al. 2002). - La méthylation est considérée comme un mécanisme de détoxification de l'As inorganique (Thompson 1993) mais il n'y a pas de consensus sur cet aspect (Cohen et al. 2006; Thomas et al. 2001). - peut affecter tous les systèmes de l'organisme (cardiovasculaire, dermatologique, nerveux, hépatobilliaire, rénal, gastro-intestinal, et respiratoire) (Tchounwou et al. 2012). - demi-vie de l'As inorganique est de 2-40 jours (Santé Canada 2006).
Pb	- peut avoir la forme organique ou inorganique (Järup 2003). - plus de 50% du Pb inorganique inhalé peut être absorbé dans les poumons (Järup 2003). - chez l'adulte, 35 à 50% de Pb sont absorbés par l'entremise de l'eau (Tchounwou et al. 2012). - son absorption est influencée par des facteurs tels que l'âge et le statut physiologique (Tchounwou et al. 2012). - chez l'adulte, 85-95% du Pb est accumulé dans les os (Flora et al. 2012) et n'est libéré que lentement de ce compartiment du corps (Järup 2003). - demi-vie dans le sang est de 28-36 jours et des décennies dans les os (ATSDR 2017). - les os contribuent de 40–70% de Pb libérés dans le sang (Flora et al. 2012). - le Pb organique (tétraméthyl et tétraéthyl), à différence de la forme inorganique, pénètre facilement la peau et il peut traverser les barrières hémato-encéphaliques chez l'adulte (Järup 2003).
Hg	- existe sous trois formes: élément métallique, sels inorganiques et composés organiques (Jaishankar et al. 2014; Tchounwou et al. 2012). - chacune présente une toxicité et une biodisponibilité différente (Jaishankar et al. 2014). Ces formes sont transformées par les microorganismes en composé organique (méthylmercure) (Jaishankar et al. 2014; Järup 2003) qui est plus toxique (Dórea et al. 2013; Health Canada 2006). - les sels inorganiques du Hg sont hydrosolubles avec une biodisponibilité de 7% à 15% après l'ingestion tandis que le Hg élémentaire provenant de l'ingestion est mal absorbé avec une biodisponibilité inférieure à 0.01%. (Park and Zheng 2012). - Hg organique (méthylmercure par exemple), environ 95% des quantités administrées par voie orale étaient absorbés dans le tube digestif selon une étude chez les humains (Aberg et al. 1969). - toutes les formes du Hg sont toxiques (Tchounwou et al. 2012). - le Hg élémentaire est principalement accumulé dans le cerveau et le rein (Park and Zheng 2012)

	- le Hg inorganique est accumulé principalement dans le rein (Park and Zheng 2012). - le méthylmercure peut facilement tranverser le placenta et les barrières hémato-encéphaliques (Tchounwou et al. 2012). - demi-vie : 30 - 60 jours dans l'organisme et 20 ans dans le cerveau (Park and Zheng 2012)
Cd	- son absorption à la suite d'une exposition est très limitée (< 6 %) (INRS 2013) et dépend si l'exposition est par voie orale (5%) (Godt et al. 2006) ou cutanée (<1%), à la différence de la rétention pulmonaire dont l'absorption est comprise entre 20 et 50 %, à la suite de l'inhalation du cadmium (INRS 2013). - son absorption gastro-intestinale est influencée par des facteurs tels que l'apport de calcium, zinc et fer. Si ces apports sont faibles cela entraîne une résorption compensatoire plus élevée du Cd grâce à son homologie moléculaire avec le calcium et le zinc ainsi que la stimulation du transporteur d'ions métalliques (DCT-1) par le faible taux sanguin du fer (Godt et al. 2006). - les reins et le foie contiennent ensemble environ 50% de l'accumulation de Cd dans le corps (ATSDR 2013) - sa demi-vie biologique est de 6-38 ans dans les reins et 4-19 ans dans le foie (ATSDR 2013) - sa présence dans le sang reflète une exposition récente tandis que la mesure urinaire indique une accumulation ou une charge du Cd dans les reins (Tchounwou et al. 2012).

2.2.2 Les sources d'exposition des métaux

Dans notre environnement, les métaux sont des composantes naturelles de la croûte terrestre. Cependant, l'utilisation anthropique des métaux dans de nombreux procédés industriels et dans les pratiques agricoles, représente la principale source des métaux dans l'atmosphère, le sol et l'eau (Huss 2011). En général, la nourriture reste la principale source du Mn (Health Canada 2016), du Hg, du Cd, de l'As (Järup 2003) et du Pb (Santé Canada 2013) suivie par la cigarette pour le Cd, par l'eau potable pour l'As (Järup 2003) et le Pb (Santé Canada 2013) et par l'amalgame dentaire pour le Hg (Health Canada 2004; Järup 2003). Les possibles sources des métaux sont detaillées dans le tableau 3 ci-dessous.

Tableau 3. Sources d'exposition aux métaux

Métaux	Sources d'exposition
Pb	Peintures, installations de plomberie au Pb, batteries (Huss 2011), aliments contaminés, jouets (Huss 2011; WHO 2016), bijoux, cosmétiques, poussière, l'eau (WHO 2016), l'air (Järup 2003) ; carburants au Pb (Huss 2011 ; MAINC 2013), exploitation minière (MAINC 2013)
Hg	Production de chlore, extraction d'or, piles, appareils de mesure, amalgames dentaires, fumée de cigarette; les poissons (espadons, thons, raies et requins) et des fruits de mer (Huss 2011), incinérateurs de déchets et les crématoires (OMS 2005)
Cd	Produits céramiques en contact avec les denrées alimentaires, milieu de travail, les cigarettes et la fumée du tabac, piles, eau potable, légumes, fruits de mer, foies et reins d'animaux (Huss 2011), joailleries et les jouets des enfants (Guney and Zagury 2014), exploitation minière, la combustion de déchets et de combustibles, la production de ciment (MAINC 2013)
As	Eau potable (dépôts minéraux naturels), légumes, notamment laitues (Huss 2011), fruits de mer (Fort et al.2014 ; Huss 2011), riz (Gilbert-Diamond et al. 2011)
Mn	la nourriture, l'eau potable, l'air (en particulier dans les expositions professionnelles), le sol (Health Canada 2016), les suppléments (gluconate de Mn, sulfate de Mn, ascorbate de Mn et chélates d'acides aminés de Mn) et les aliments (grains entiers, noix, légumes à feuilles et thés) (Linus Pauling Institute 2014)

2.2.3 Les niveaux d'exposition aux métaux au Canada

Au Canada, des études ont rapporté des niveaux sanguins des métaux (Pb, Hg et Mn) au niveau de la mère ou dans le cordon ombilical chez les premières nations Cris (Hanning et al. 2003) et les Inuits (Levesque et al. 2003), ainsi que dans la population maritime (Belles-Isles et al. 2002), la population montréalaise (Smargiassi et al. 2002) et le sud du Québec (Lafond et al. 2004; Rhainds et al. 1999; Takser et al. 2004) (voir tableau 4). L'étude de Wong and Lye (2008) a montré cependant que les niveaux sanguins des métaux (en particulier le Pb) dans la population générale ont considérablement diminué au Canada par rapport à ceux mesurés entre 1978 et 1979 (grâce à l'élimination du Pb dans l'essence). Malgré cette diminution, le Pb demeure un problème de santé publique important en raison de son accumulation et sa persistance dans les os (Wong and Lye 2008).

L'enquête canadienne sur les mesures de la santé (Wong and Lye 2008) a montré que les moyennes géométriques des concentrations sanguines de Pb, de Hg total et de Cd étaient

respectivement de 1.37µg/dl, 0.76µg/l et 0.35µg/l. La limite de détection (LDD) était de 0.02µg/dl pour le Pb, de 0.10µg/l pour le Hg et de 0.04µg/l pour le Cd. Ainsi, chez la population de 6 à 79 ans, 99% étaient au-dessus de la LDD pour le Pb, 90% pour le Hg total et 98 % pour le Cd. Les résultats ont montré que moins de 1% de la population canadienne de 6 à 79 et de 20 à 79 ans étaient respectivement au-dessus des valeurs limites recommandées par Santé Canada pour le Pb (10 µg/dl) et le Hg (20 µg/l) (Wong and Lye 2008). Il n'y a pas encore de valeur limite établie par Santé Canada pour le Cd. Les niveaux d'exposition aux métaux documentés au sein d'étude réalisée au Canada, aux États-Unis et en Allemagne sont présentés dans le tableau 4.

Tableau 4. Comparaison des moyennes géométriques (MG) des concentrations sanguines de Pb, de Hg total et de Cd dans la population générale du Canada, des États-Unis et de l'Allemagne.

	ECMS		NHANES (2001 à 2002)		GerES (1998 et 2003-2006)		INSPQ (2004)	
	Âge (années)	MG	Âge (années)	MG	Âge (années)	MG	Âge (années)	MG
Pb (µg/dl)	6-19	0.88	6-11 12-19	1.25 0.94	6-8 9-11 12-14	1.73 1.56 1.45		
	20-79	1.50	≥20	1.56	18- 69	3.07	18-65	2.15
Hg total (µg/l)	6-19	0.31E			6-8 9-11 12-14	0.23 0.22 0.26		
	20-79	0.91E	16-49 femmes	0.83	18- 69	0.58	18-65	0.74
Cd (µg/l)	6-19	0.15	6-11 12-19	< 0.3 < 0.3	6-8 9-11 12-14	< 0.12 < 0.12 0.14		
	20-79	0.42	≥20	< 0.3	18-69	0.58	18-65	0.69

Source: Wong SL, Lye EJD. 2008. Niveaux de plomb, de mercure et de cadmium chez les Canadiens. Statistique Canada: Rapports sur la santé. 19(4): 1-8.
Remarque des auteurs : E= à utiliser avec prudence (coefficient de variation de16.6 % à 33.3 %)
MG = moyennes géométriques
ECMS = Enquête canadienne sur les mesures de la santé, 2007-2008
NHANES = National Health and Nutrition Examination Survey des États-Unis
GerES = German Environmental Survey III et IV
INSPQ = Institut national de santé publique du Québec

Il existe peu de données sur les niveaux de métaux chez les femmes enceintes canadiennes. Quelques études faites au Québec et en Ontario ont rapporté des niveaux de Pb (Hanning et al. 2003; Smargiassi et al. 2002), de Hg (Rhainds et al. 1999) et de Mn (Smargiassi et al. 2002; Takser et al. 2004) chez les femmes enceintes canadiennes (présenté au tableau 5).

Tableau 5. Niveaux de métaux chez les femmes enceintes rapportés par les études canadiennes

Auteurs	Populations	Prélèvements	Métaux	Niveaux moyens/ géométriques
Hanning et al. 2003	Premières nations Cris	Sang maternel Cordon ombilical	Pb	0.11±0.06 µmol/l (2.28±1.24 µg/l) 0.10±0.08 µmol/l (2.07±1.66 µg/l)
Levesque et al. 2003	Inuit	Cordon ombilical	Pb	≥ 0.48 µmol/l (9.95 µg/l)
Belles-Isles et al. 2002	Population maritime (groupe de la pêche de subsistance / référence)	Cordon ombilical	Pb Hg	1.64 µg/l (1.89, 1.91) / 1.33 (1.22, 1.47) 0.0018 µg/l (0.0014, 0.0023) / 0.0009 (0.0008, 0.0010)
Smargiassi et al. 2002	Montréal	Sang maternel Cordon ombilical Sang maternel Cordon ombilical	Pb Mn	2.1 µg/dl 1.7 µg/dl 23±13 µg/l 45±20 µg/l
	Paris	Sang maternel Cordon ombilical Sang maternel Cordon ombilical	Pb Hg	5.4 µg/dl 3.2 µg/dl 23±15 µg/l 42±15 µg/l
Rhainds et al. 1999	Québec	Cordon ombilical	Pb Hg	1.575 µg/l (1.533, 1.637) 0.001 µg/l (0.0009, 0.0010)
Takser et al. 2004	Sud-ouest du Québec	Sang maternel Cordon ombilical	Mn	15.6 µg/l 32.3 µg/l

2.2.4 Les effets des métaux sur la santé

- **Chez la population générale**

Les métaux peuvent avoir des effets néfastes sur la santé. Le tableau 6 décrit les effets rapportés sur la santé dans la population générale.

Tableau 6. Effets des métaux sur la santé dans la population générale

Métaux	Effets rapportés sur la santé
Pb	Atteintes rénales, dépressions allant jusqu'au suicide, manque d'attention, atteinte aux fonctions motrices, troubles de mémoire, difficultés d'apprentissage, états de fatigue, agitation, agressivité, psychoses, hallucinations, polyneuropathie périphérique, encéphalopathie, saturnisme (Huss 2011), accident vasculaire cérébral, infarctus du myocarde (GOC 2013), hypertension artérielle (Huss 2011; GOC 2013; OMS 2017), coma, convulsions, anémie (OMS 2017)
Hg	Maladies auto-immunes (arthrite rhumatoide, lupus, sclérose en plaques), maladies cardiovasculaires, cancer du foie, agitation, agressivité, troubles visuels et auditifs, polyneuropathie, myasthénie grave, engourdissement et des picotements aux extrémités, vision trouble (Huss 2011), hypertension artérielle, surdité, manque de coordination musculaire (GOC 2006 ; Huss 2011), troubles de l'attention et retard du développement chez l'enfant, tremblements, paralysie, insomnie, instabilité émotionnelle (Huss 2011; OMS 2005), lésions pulmonaires (OMS 2005), éruptions cutanées, transpiration excessive, irritabilité, fibrillation musculaire, crises d'épilepsie (GOC 2006)
Cd	Lésions rénales et pulmonaires, fragilisation des os avec une diminution de la densité minérale osseuse, anémies, cancer du poumon (Huss 2011), dysfonctionnement pulmonaire mortel (Huss 2011; Wong and Lye 2008), cancer du poumon, l'hypertension artérielle (Wong and Lye 2008), vomissements, diarrhée (ATSDR 2012a; Santé Canada 2008), cancer du poumon (ATSDR 2012a), l'oedème pulmonaire, maux de tête, nausées, frissons, l'emphysème pulmonaire, calculs rénaux (Santé Canada 2008)
As	Diabète de type 2, surdité, paresthésies, psychoses organiques avec somnolence, stupeur, délires, schizophrénie (Huss 2011), hypertension artérielle (Abhyankar et al. 2012), vomissements, nausées, diarrhée (ATSDR 2007; OMS 2016 ; Santé Canada 2017), douleurs abdominales, cancer de la peau, de la vessie, des poumons (ATSDR 2007; OMS 2016) et du foie (ATSDR 2007)
Mn	A doses normales (4–15 µg/l dans le sang, 1–8 µg/l dans l'urine et 0.4–0.85 µg/l dans le sérum (ATSDR 2012b)): la formation des os, l'intégrité de la peau, le contrôle de la glycémie, la fonction antioxydante et les interactions avec d'autres nutriments (fer, magnésium et calcium) (Linus Pauling Institute 2014 ; WHF 2014) A doses élevées (ou surdose) : neurotoxicité (maladie de Parkinson, l'irritabilité, l'agressivité, hallucinations), réponse inflammatoire des poumons (la toux, la bronchite aiguë et une diminution de la fonction pulmonaire) (Linus Pauling Institute 2014), hypertension artérielle (Lee and Kim 2011) A faibles doses (déficience) : possibles maladies chroniques (diabètes mellitus, épilepsie, ostéoporose), croissance altérée, altération de la fonction de reproduction (Linus Pauling Institute 2014), anomalies squelettiques (Linus Pauling Institute 2014; WHF 2014)

- **Chez la femme enceinte** :

Les métaux peuvent aussi représenter un risque au bon déroulement de la grossesse (Vigeh et al. 2008; SESST 2013; Wood 2009). Le tableau 7 décrit les effets rapportés chez les femmes enceintes et leurs bébés.

Tableau 7. Effets probables des métaux sur la santé des femmes enceintes et l'évolution de la grossesse

Métaux	Effets possibles pour les femmes enceintes et leurs fœtus
As	Effet foetotoxique (augmentation des malformations du cerveau, des yeux, des os et parfois des reins et des gonades), effet mutagène (SESST 2013), avortements spontanés et mortinaissances (Amadi et al. 2017)
Cd	Faible poids à la naissance (Ikeh-Tawari et al. 2013; Kippler et al. 2012; Romano et al. 2016), accouchement prématuré (Nishijo et al. 2002), avortements spontanés et mortinaissances (Amadi et al. 2017)
Hg	Mutagène, peut avoir certains effets foetotoxiques (SESST 2013), retard de croissance fœtal (OMS 2005), avortements spontanés et mortinaissances (Amadi et al. 2017)
Pb	Carcinogène, effet morphologique et neurologique, faible effet mutagène et tératogène, augmentation de mortinaissance, retard de croissance natale et post-natale (SESST 2013), faible poids de naissance (ACOG 2012, GOC 2013 ; NCEH 2010), prématurité (NCEH 2010, GOC 2013 ; SESST 2013), augmentation d'avortement spontané (ACOG 2012, GOC 2013 ; NCEH 2010, SESST 2013)
Mn	Effets bénéfiques : fonctions essentielles pour la santé maternelle et le développement fœtal (Gluckman et al. 2015) grâce à ses actions métaboliques (le développement des os et du tissu connectif, l'activation des enzymes antioxydantes et régulatrices du métabolisme des carbohydrates, lipides et protéines) (Avila et al. 2013) Effets adverses : - Concentration sanguine maternelle de Mn faible : retard de croissance intra-utérin (Vigeh et al. 2008), - Concentration urinaire maternelle de Mn élevée : faible poids de naissance (Xia et al. 2016) - Concentration sérique du cordon ombilical de Mn élevée: pauvre développement neurocomportemental du fœtus (Yu et al. 2014)

2.2.5 Les effets des métaux sur la TA

La section qui suit présente les études sur l'association entre les métaux et l'hypertension. L'annexe 2 présente aussi un tableau détaillé de chacune de ces études effectuées dans la population générale ou chez les femmes enceintes.

- **Études chez les animaux**

Des études effectuées chez des rats ont permis de mettre en évidence une relation positive entre le Pb (0.1-5.0 ppm) et la TA élevée (Perry et al. 1988). Les mêmes observations ont été rapportées pour le Cd (0.5-1.0 mg%) (Walker and Moses 1979) et l'As (100 parts per billion (ppb)) (Sanchez-Soria et al. 2012). Par contre, un effet protecteur sur la TA a été observé chez les rats exposés au Mn (10 mg/kg) (Muhammad et al. 2012). Une autre étude chez les rats a montré que

le méthylmercure (1.0 µg/kg) augmente la TA, contrairement au Hg inorganique (1.3 µg/kg) où il n'y avait pas d'évidence d'une association avec la TA (Wildemann et al. 2015). De Assis et al. (2003) ont aussi trouvé dans leur étude chez les rats qu'une exposition continue à de faibles concentrationss de Hg (5 et 20 nmol) peut affecter la fonction cardiaque avec une réduction de la pression systolique et une augmentation de la pression diastolique.

- **Études dans la population générale**

Le tableau 8 donne un résumé de la nature de l'association (positive, négative ou une absence d'association) des différentes études effectuées dans la population générale.

Le Mn et la TA

Le Mn a des fonctions physiologiques (Avila et al. 2013) contrairement aux autres métaux (Jaishankar et al. 2014). Des liens ont été mis en évidence entre le Mn et la TA. Par exemple, Mordukhovich et al. (2012) ont observé dans leur étude transversale (n = 639 hommes) une diminution de la TA associée au Mn (beta = -1.09 mmHg; 95% IC: -2.08, -0.10 pour TAS et -0.62 mmHg; 95% IC: -1.15, -0.09 pour la TAD et -0.47mmHg; 95% IC :-1.26, 0.32 pour la pulsation de pression, $p < 0.05$). Contrairement à l'étude de Mordukhovich et al. (2012), Lee and Kim (2011) ont trouvé dans une étude transversale (n = 2005 hommes et femmes) une association entre le Mn et le risque accru d'hypertension artérielle (OR = 1.567; 95% IC : 1.167, 2.103). D'autre part, il est possible que la relation entre le Mn et l'hypertension artérielle puisse être modifiée par le sexe car, Lee et al. (2015) ont observé une réduction de la TAS associée à la consommation du Mn chez les hommes et non chez les femmes. Nous pouvons constater que la littérature n'est pas cohérente sur la relation entre le Mn et la TA car certaines études ont mis en évidence des associations négatives et d'autres études, des associations positives.

L'As et la TA

L'association entre l'As et la TA a été mise en évidence par certaines études. Dans une étude transversale (n = 639 hommes), Mordukhovich et al. (2012) ont détecté une association positive entre l'As et la TA (beta = 0.93 mmHg; 95% IC: 0.25, 1.62 pour la TAS, la pulsation de pression (TAS-TAD) de 0.76 mmHg; 95% IC: 0.22, 1.30 et 0.17 mmHg; 95% IC :-0.20, 0.55 pour la TAD; $p < 0.05$). D'autres auteurs ont aussi mis en évidence une association positive entre l'As et la TA (Abhyankar et al. 2012; Yu et al. 2017). Selon cette revue de la littérature, la relation entre l'As et la TA semble être positive dans la population générale.

Le Cd et la TA

Contrairement à l'étude de Mordukhovich et al. (2012) qui n'a pas trouvé d'association entre le Cd et la TA, Tellez-Plaza et al. (2008) ont observé dans une étude transversale (n = 10991 hommes et femmes) un lien entre le Cd sanguin et la TAS (beta = - 1.36 mmHg 95% IC : -0.28, 3.00, $p < 0.05$) et la TAD (beta = 1.68 mmHg; 95% IC : 0.57, 2.78, $p < 0.05$). D'autres auteurs ont aussi rapporté une absence de lien entre le Cd et la TA (Al-Saleh et al.2006; Shiue and Hristova 2014). Ces résultats suggèrent que la relation entre le Cd et la TA n'est pas clairement définie.

Le Hg et la TA

Dans la littérature, l'association entre le Hg et la TA a été identifiée comme positive, négative ou absente. Par exemple, dans une étude transversale (n = 251 femmes et hommes), Fillon et al. (2006) ont rapporté un lien positif entre le Hg mesuré dans les cheveux et la TAS ≥ 130 mmHg (OR = 2.91; 95% CI : 1.26, 7.28). Par contre, le Hg mesuré dans l'urine a été associé à un risque réduit pour l'hypertension artérielle (OR = 0.87; 95% CI : 0.78, 0.99) (Park et al. 2013). Dans leur étude de cohorte (n = 262 femmes et hommes), Goodrich et al. (2013) n'ont pas trouvé

d'association entre le Hg élémentaire mesuré dans l'urine (moyenne = 0.94 µg/l) et la TAS contrairement au méthylmercure mesuré dans les cheveux (moyenne = 0.45 µg/g) qui était associé à une augmentation de la TAD. Mordukhovich et al. (2012) n'ont pas aussi trouvé d'association entre le Hg mesuré dans les ongles des orteils et la TA. D'autre part, il est possible que la relation du Hg avec l'hypertension artérielle puisse être modifiée par le sexe. Nielsen et al. (2012) ont observé dans leur étude transversale (n = 1861 femmes et hommes) un lien entre le Hg et la réduction de la TAD chez les hommes mais pas chez les femmes (beta = -0.04; $p < 0.05$).

Le Pb et la TA

Quant au Pb, il est également possible que sa relation avec l'hypertension artérielle soit modifiée par le sexe. Par exemple une association positive a été observée avec la TAD (beta = 2.36; 95% IC : 0.94, 3.79, $p < 0.05$) chez les hommes mais pas chez les femmes (Bushnik et al. 2014). Mordukhovich et al. (2012) n'ont pas trouvé d'association entre le Pb et la TA contrairement à Lee et al. (2016) qui ont mis en évidence des associations positives.

Tableau 8. Études sur l'association entre les métaux et la tension artérielle (TA) ou l'hypertension artérielle dans la population générale en fonction du type d'association trouvée (positive, négative ou une absence d'association)

Métal		Études ayant montré une association positive	Études ayant montré une absence d'association	Études ayant montré une association négative
As	As (mesuré dans différents échantillons corporels ou l'eau)	Mordukhovich et al. 2012 Hall et al. 2017 Abhyankar et al. 2012 Yu et al. 2017 Afridi et al. 2015		
	Les espèces de l'As urinaire	Li et al. 2013 Shiue 2014a Shiue 2014b	Jones et al. 2011 Li et al. 2013	
Pb (mesuré dans différents échantillons corporels)		Bushnik et al. 2014 Lee et al. 2016 Afridi et al. 2015	Mordukhovich et al. 2012 Bushnik et al. 2014 González-Muñoz et al. 2010	
Hg (mesuré dans différents échantillons corporels)		Goodrich et al. 2013 Bautista et al. 2009 Fillion et al. 2006 Vupputuri et al. 2005	Mordukhovich et al. 2012 Park et al. 2013 Nielsen et al. 2012 Bautista et al. 2009 Fillion et al. 2006 Vupputuri et al. 2005	Park et al. 2013 Nielsen et al. 2012 Goodrich et al. 2013 Pedersen et al. 2005
Cd (mesuré dans différents échantillons corporels)		Eum et al. 2008 Tellez-Plaza et al. 2008 Afridi et al. 2013 Lee et al. 2016	Mordukhovich et al. 2012 Al-Saleh et al. 2006 Shiue et Hristova 2014	Tellez-Plaza et al. 2008
Mn (mesuré dans différents échantillons corporels)		Lee and Kim 2011		González-Muñoz et al. 2010 Lee et al. 2015 Kim and Choi 2013 Mordukhovich et al. 2012

- **Études chez les femmes enceintes**

La majorité des études publiées concernant l'exposition aux métaux et la TA chez les femmes enceintes portent sur la relation entre l'exposition au Pb et le risque des désordres hypertensifs de la grossesse. Par ailleurs, le tableau 9 donne un résumé des études effectuées chez les femmes enceintes en fonction de leur trouvaille sur la nature de l'association (positive, négative ou une absence d'association).

Tableau 9. Études sur l'association entre les métaux et la TA ou les désordres hypertensifs de grossesse chez les femmes enceintes en fonction du type d'association trouvée (positive, négative ou une absence d'association)

Métal		Études ayant montré une association positive	Études ayant montré une absence d'association	Études ayant montré une association négative
As	As sanguin		Maduray et al. 2017 Sandoval-Carrillo et al. 2016	
	As urinaire	*Farzan et al. 2015		
Pb (sanguin ou sérique)		Magri et al. 2003 *Rabinowitz et al. 1987 Motawei et al. 2013 Ikechukwu et al. 2012 Sowers et al. 2002 *Wells et al. 2011 Vigeh et al. 2006 Yazbeck et al. 2009 Jameil 2014	Maduray et al. 2017	
Hg sanguin		*Wells et al. 2017 Pan et al. 2007		*Wells et al. 2017
Cd sanguin		Kosanovic et Jokanovic 2007 Kosanovic et al. 2002 Laine et al. 2015	Yazbeck et al. 2009 Lazebnik et al. 1989	
Mn (sanguin ou sérique)		Vigeh et al. 2006 Vigeh et al. 2013	Yazbeck et al. 2009	Sarwar et al. 2013 Al-Jameil et al. 2014 Maduray et al. 2017

*études ayant montré une association en considérant la TA (et non les désordres hypertensifs de la grossesse).

L'exposition au Mn et les désordres hypertensifs de la grossesse

Les résultats des études sur l'association entre le Mn et les désordres hypertensifs de la grossesse ne sont pas consistents. Certaines études ont mis en évidence des associations positives tandis que d'autres ont montré des associations négatives ou une absence d'association. L'étude cas-témoins de Vigeh et al. (2006), a observé une association entre la prééclampsie et les concentrations du Mn dans le sang du cordon. Les niveaux moyens du Mn dans le cordon ombilical étaient significativement plus élevés dans les cas de prééclampsie (46.87 ± 15.03 µg/l) comparativement aux contrôles (40.32 ± 15.19 µg/l). Dans une autre étude (Vigeh et al. 2013),

les concentrations sanguines de Mn mesurées au 1er et 2ième trimestre de grossesse étaient significativement plus élevées chez les femmes enceintes (n = 364) ayant développé l'hypertension gestationnelle que chez les femmes enceintes normotendues (OR = 47.0; 95% IC : 4.0, 556.4 et OR = 5.5; 95% IC : 1.1, 29.0, respectivement). Contrairement aux résultats des deux études précédentes, Sarwar et al. 2013 ont observé des concentrations sanguines moyennes du Mn plus faibles chez des femmes prééclamptiques (0.08 ± 0.02 µg/l) que chez les contrôles (0.14 ± 0.02 µg/l) p <0.05. D'une manière similaire, dans leur étude cas-témoins (n = 120 femmes enceintes) Al-Jameil et al. (2014) ont observé les niveaux moyens du Mn plus faibles chez les femmes prééclamptiques (0.072 ± 0.06 µg/l) par rapport aux contrôles (0.125 ± 0.07 µg/l). Maduray et al. (2017) ont aussi rapporté dans leur étude cas-témoins (n = 66 femmes enceintes) des niveaux moyens du Mn dans le sérum significativement plus bas chez les femmes prééclamptiques (0.02 ± 0.0 µg/l) par rapport aux contrôles (0.03 ± 0.0 µg/l), p=0.03. Ces études mettent en lumière le fait que le Mn peut être associé aux désordres hypertensifs de la grossesse à des doses élevées ou faibles. Cela semble en lien avec le maintien de niveaux adéquats de Mn (doses normales : 4–15 µg/l dans le sang, 1–8 µg/l dans l'urine et 0.4–0.85 µg/l dans le sérum (ATSDR 2012) afin de réduire les risques des désordres hypertensifs de la grossesse. Par contre, l'étude de Yazbeck et al. (2009), n'a pas observé d'association entre le Mn et l'hypertension gestationnelle.

L'exposition au Cd et les désordres hypertensifs de la grossesse

Les concentrations sanguines du Cd chez les fumeurs sont quatre à cinq fois plus élevées par rapport aux non-fumeurs (Kosanovic and Jokanovic 2007). L'exposition à ce métal a été associée aux désordres hypertensifs de la grossesse (Kosanovic and Jokanovic 2007; Laine et al. 2015)

quoique les études dans la littérature sur cette association soient divergentes. Certaines études ont montré une association positive entre le Cd et les désordres hypertensifs de la grossesse (Kosanovic and Jokanovic 2007; Laine et al. 2015) tandis que d'autres n'ont pas trouvé d'association (Maduray et al. 2017; Yazbeck et al. 2009). D'autre part, il est possible que la relation entre le Cd et l'hypertension gestationnelle puisse être modifiée par le tabagisme maternel (Kosanovic and Jokanovic 2007). Dans une étude cas-témoins, le niveau moyen du Cd dans le sang maternel était significativement plus élevé chez les participantes hypertendues fumeuses (1.9 ± 0.6 µg/l) que celui des hypertendues et non fumeuses (1.3 ± 0.1 µg/l) et des normotendues et non fumeuses (0.8 ± 0. 3 µg/l) (Kosanovic and Jokanovic 2007). Les mêmes observations ont été rapportées par Kosanovic et al. 2002. Ces deux études ont mis l'accent sur le rôle potentiel du Cd dans l'étiologie des désodres hypertensifs de la grossesse chez les femmes enceintes fumeuses ayant une déficience en sélénium (Kosanovic et al. 2002; Kosanovic and Jokanovic 2007) (un antioxydant qui protège contre la toxicité du Cd à travers la réduction du stress oxydatif induit par Cd (Wu et al. 2016)). A l'inverse, aucune différence dans les niveaux d'exposition de Cd dans le sang maternel et dans le placenta n'a été observée chez 43 patientes hypertendues et leurs contrôles dans l'étude de Lazebnik et al. (1989). Une étude cas-témoins nichée auprès de 172 femmes enceintes, a révelé que les niveaux placentaires de Cd (3.6 ng/g, allant de 0.52 à 14.5 ng/g) étaient associés à une augmentation du risque de prééclampsie (OR = 1.5; 95% IC: 1.1, 2.2) (Laine et al. 2015). Par ailleurs, l'étude de Yazbeck et al. (2009) n'a pas observé d'association entre le Cd sanguin maternel (0.9 ± 0.5µg/l) et l'hypertension gestationnelle tout comme l'étude de Maduray et al. (2017) qui n'a pas observé de lien entre le Cd mesuré dans le sérum maternel (0.05±0.04 µg/l) et la prééclampsie. Cette absence

d'association pourrait s'expliquer par les faibles niveaux du Cd dans les deux dernières études.

L'exposition au Pb et les désordres hypertensifs de la grossesse

Quelques études ont observé une relation entre l'exposition au Pb et les désordres hypertensifs de la grossesse. En Égypte, une étude transversale (Motawei et al. 2013) réalisée chez 140 femmes enceintes a montré une association significative entre la plombémie maternelle mesurée durant la grossesse et la prééclampsie. Dans cette étude la plombémie moyenne mesurée chez les 115 femmes enceintes prééclamptiques était 2.5 fois plus élevée que celle des femmes enceintes non prééclamptiques (moyenne = 37.68 ± 9.17 µg/dl versus 14.5±3.18 µg/dl, $p < 0.001$). Des résultats similaires ont été observés dans une étude réalisée chez un groupe de femmes enceintes nigérianes (n = 59) prééclamptiques et leurs contrôles (n = 150) pour lesquelles les plombémies moyennes mesurées durant la grossesse étaient respectivement de 60.2 ± 12.8 µg/dl et de 26.3 ± 8.0, $p < 0.001$ (Ikechukwu et al. 2012). Jameil (2014) a mis en évidence des niveaux sériques moyens de Pb plus élevés chez les femmes prééclamptiques (27.18 ± 2.13 µg/dl) par rapport aux contrôles (18.23 ± 2.34 µg/dl). Contrairement aux précédentes études où les niveaux de Pb étaient très élevés chez les femmes prééclamptiques, d'autres auteurs ont mis en évidence des associations avec des niveaux plus faibles du Pb. Dans une étude de cohorte de femmes enceintes (n = 285) réalisée aux États-Unis, une association positive a été observée entre les niveaux de Pb mesurés dans le sang ombilical (moyenne géométrique faible = 0.66 µg/dl) et la TA maternelle (Wells et al. 2011). De même, Sowers et al. (2002) ont observé chez 705 femmes enceintes, une association significative entre le Pb maternel (moyenne de 1.2 ± 0.03 µg/dl) et la prééclampsie. L'étude cas-témoins (Vigeh et al. 2006) réalisée en Iran chez 396 femmes sans antécédent d'exposition professionnelle aux métaux a montré que les niveaux moyens de Pb dans le cordon

ombilical étaient significativement plus élevés dans les cas de prééclampsie (4.30 ± 2.49 µg/dl) comparativement aux contrôles (3.52 ± 2.09 µg/dl). Par ailleurs, une étude de cohorte réalisée sur 1017 femmes enceintes françaises a montré que les niveaux sériques moyens de Pb au 2^{ième} et 3^{ième} trimestre de grossesse chez les femmes enceintes qui ont développé l'hypertension gestationnelle étaient de 2.2 ± 1.4 µg/dl comparativement à 1.9 ± 1.2 µg/dl, p = 0.02 chez les femmes n'ayant pas eu d'hypertension (Yazbeck et al. 2009). Malgré que la différence soit statistiquement significative dans cette étude, nous pouvons constater que l'écart entre les niveaux du Pb chez les femmes hypertendues et normotendues était faible. Dans une autre étude transversale (n = 143 femmes enceintes), les concentrations moyennes du Pb étaient plus élevées chez les femmes avec l'hypertension gestationnelle (9.6 ± 6 µg/dl) par rapport aux contrôles (5.8 ± 3 µg/dl), p = 0.002 (Magri et al. 2003). Dans une étude cas-témoins (n = 165 femmes enceintes), Vigeh et al. (2004) ont observé des niveaux moyens du Pb plus élevés chez les femmes avec hypertension gestationnelle globale (5.7 ± 2 µg/dl) que chez les normotendues (4.8 ± 1.9 µg/dl), p <0.05. Une corrélation positive a été trouvée entre les niveaux du Pb et la TAS (corrélation de Pearson (r) = 0.081, p < 0.001) et la TAD (r = 0.051, p = 0.002) (Rabinowitz et al. 1987). Les niveaux moyens du Pb dans cette étude étaient de 7.6 ± 0.2 µg/dl chez les hypertendues et de 6.9 ± 0.1 µg/dl chez les normotendues, p = 0.0013 (Rabinowitz et al. 1987). Par contre Maduray et al. (2017) n'ont pas observé de différence entre les niveaux moyens du Pb dans le sérum des femmes prééclamptiques sud-africaines (0.20 ± 0.17 µg/l) par rapport à leurs contrôles (0.16 ± 0.21 µg/l), p = 0.22. Cette étude récente se démarque cependant des autres études avec des niveaux mesurés de Pb qui sont beaucoup plus faibles. Cela pourrait expliquer l'absence d'association trouvée dans cette étude.

L'exposition au Hg et les désordres hypertensifs de la grossesse

Les études concernant l'association entre le Hg et les désordres hypertensifs de la grossesse sont très rares. Dans l'étude transversale de Wells et al. (2017) (n = 263 femmes enceintes), le Hg inorganique (MG (95% IC) = 0.13 µg/l (0.10, 0.17)) a été associé à une TAS et une pression d'impulsion plus faible alors que le méthylmercure (0.95 µg/l (0.84, 1.07)) a été associé à une TAS plus élevée (Wells et al. 2017). Cette étude met en évidence que les différentes espèces du Hg peuvent avoir des rapports différents avec la TA, le méthylmercure étant considéré comme la forme du Hg la plus toxique (Hong et al. 2012). D'autres études sont nécesssaires pour confirmer ces résultats.

L'exposition à l'As et les désordres hypertensifs de la grossesse

Les études examinant l'association entre l'As et les désordres hypertensifs de la grossesse sont très rares. En plus, celles que nous avons recensées rapportent des résultats divergents. Une a montré une association positive tandis que l'autre n'a pas trouvé d'association. En utilisant les données provenant de l'étude de cohorte de naissance du New Hampshire, dont 514 femmes enceintes, Farzan et al. (2015) ont observé que chaque augmentation de 5µg/l de l'As mesuré dans l'urine était associée à une augmentation de 0.15 mmHg de la TAS, p = 0.022. Par contre Maduray et al. (2017), n'ont pas mis en évidence de différence significative entre les niveaux de l'As mesurés dans le sérum maternel chez les normotendues (0.49 ± 0.0 µg/l) et les prééclamptiques (0.06 ± 0.0 µg/l), p = 0.81. Cette absence d'association pourrait s'expliquer par le fait que l'issue considérée était la prééclampsie et non la TA. Et par les faibles niveaux de l'As dans l'étude de Maduray et al. (2017) comparativement à celle de Farzan et al. (2015). D'autres études sont aussi nécessaires afin d'élucider cette association.

2.3 Les amalgames dentaires

Les amalgames dentaires sont utilisés depuis plus de 150 ans (FDA 2015) et sont constitués de 50% de Hg élémentaire et un alliage en poudre composé d'argent, d'étain et de cuivre (FDA 2015). La proportion des éléments de cet alliage en poudre est de 35% pour l'argent, 9% pour l'étain, 6% pour le cuivre (ATSDR 2015; Lorscheider et al. 1995). On parle aussi de trace de zinc (ATSDR 2015; Lorscheider et al. 1995). Les mouvements de mastication ou la corrosion des amalgames dentaires provoquent la libération continue de petites quantités de Hg dans la salive. De cette manière, on estime que 3 à 17 μg de Hg inorganique sont liberés par jour selon le nombre présent d'amalgames dentaires (ATSDR 2015). Chez la femme enceinte, les niveaux de Hg dans le sang sont variables (Vahter et al. 2000; Palkovicova et al. 2008). On estime que 6.4% des niveaux de Hg chez la femme enceinte sont attribués aux amalgames dentaires alors que 8.75% proviendraient de la consommation des produits de mer (Golding et al. 2016).

Chez les femmes enceintes, le Hg provenant de l'amalgame dentaire apparaitraît dans le sang maternel et fœtal et le liquide amniotique dans les 2 jours suivant la mise en bouche de ce dernier durant la grossesse (Vimy et al. 1990). L'accumulation du Hg durant la grossesse progresse dans les tissus maternels et fœtaux avec l'avancement de la gestation (Vimy et al. 1990). Le Hg libéré par l'amalgame dentaire n'a aucune fonction physiologique dans le corps (Jaishankar et al. 2014) et pourrait affecter la santé (Akbal et al. 2014; Kern et al. 2014; Méndez-Visag 2014; Zwicker et al. 2014). Cependant, les études dans la population générale ne sont pas concordantes (Ahlqwist et al. 1993; Siblerud 1990). Siblerud (1990) a trouvé dans son étude transversale (n = 101 hommes et femmes), une augmentation de la TA (TAS = 106.44 ±9.5 et TAD = 63.04±7.91, $p<0.05$) chez les personnes exposées aux amalgames dentaires par rapport

aux non-exposées (TAS=100.71±11.48 et TAD=58.67±8.22). Par contre, d'autres études n'ont pas observé de lien entre les amalgames dentaires et les maladies cardiovasculaires telles que l'infarctus du myocarde chez les femmes (Ahlqwist et al. 1993; Bengtsson et al. 2001). Nous n'avons pas trouvé d'études chez les femmes enceintes examinant la relation entre les amalgames dentaires et les désordres hypertensifs de la grossesse ou la TA. Les études sur le lien entre les amalgames dentaires et les maladies cardiovasculaires réalisées dans la population générale sont décrites dans le tableau 38 dans l'annexe 2. Comme c'est possiblement par l'entremise de l'exposition au Hg que les amalgames dentaires puissent être associés aux désordres hypertensifs de la grossesse, la littérature présentée plus haut sur l'association entre le Hg et les désordres hypertensifs de la grossesse est aussi pertinente.

2.4 Le Bisphénol A (BPA) et le triclosan (TCS)

2.4.1 Les caractéristiques du BPA et du TCS

Le BPA est un produit chimique industriel utilisé dans la fabrication des plastiques durs et transparents connus sous le nom de polycarbonate (GOC 2017). Il est biologiquement actif (Gassman et al. 2015). Le BPA est excrété dans l'urine (Gonzalez-Parra et al. 2013; Liao et Kannan 2012). Pour évaluer l'exposition humaine au BPA, on utilise habituellement le BPA urinaire total (libre et conjugué) (Garcia-Prieto et al. 2008). La forme glucuronide du BPA est la forme prédominante excrétée dans l'urine (Provencher et al. 2014). La conjugaison du BPA chez l'humain avec le glucuronide ou le sulfate est considérée comme un mécanisme de détoxification (Ginsberg and Rice 2009; Pastor-Belda et al. 2016). D'autre part, il a été rapporté que la forme glucuronide du BPA peut être déconjuguée dans l'utérus par la bêta-glucuronidase qui est une enzyme trouvée dans le placenta, le foie, les reins et l'intestin (Ginsberg et Rice 2009). Le BPA

peut être détecté dans le sérum, l'urine (Liao and Kannan 2012; Teeguarden et al. 2016), le décidua ou les villosités chorioniques (Chen et al. 2016), le liquide amniotique (Edlow et al. 2012), le sang maternel, le sang de cordon (Yamamoto et al. 2016) et le tissu placentaire (Troisi et al. 2014).

L'exposition au BPA se produit couramment par la voie orale (90%) et par la peau (10%) (von Goetz et al. 2017). Le BPA est le monomère des polycarbonates le plus fréquemment utilisé dans la préparation et la conservation des aliments (Health Canada 2014). Il est utilisé dans les résines époxydes, qui servent de revêtement protecteur à l'intérieur des boîtes à base de métal pour les aliments et les boissons. Il est également utilisé dans de nombreux produits de consommation (FDA 2016b; GOC 2017), y compris des bouteilles d'eau réutilisables (GOC 2017).

L'humain est aussi largement exposé au triclosan (TCS) dont l'utilisation s'est répandue depuis 1972 (Bergstrom 2014). Le TCS est un produit chimique utilisé comme agent de conservation ainsi qu'agent antimicrobien dans divers produits tels que les savons, lotions, parfums, déodorants, nettoyants pour la peau, maquillage des yeux et du visage, produits de santé naturels, nettoyeurs et détergents à usage général, lavages corporels, gels de douche et shampooings, médicaments sans ordonnance (comme les dentifrices, le rince-bouche et le désinfectant pour les mains) (GOC 2016). On le trouve également dans les vêtements, les ustensiles de cuisine, les meubles et les jouets (FDA 2016a). Par contre, le TCS n'a pas de sources naturelles connues et sa présence dans l'environnement est exclusivement due à l'activité anthropique (ECCC 2017).

Le TCS peut subir des transformations conduisant à la formation des composants toxiques

(Bedoux et al. 2012). Il est transformé en phénols chlorés et éthers biphényliques, en méthyl triclosan et en dibenzodioxines chlorées après chloration, méthylation biologique et photo-oxydation, respectivement. Ces substances sont des composés persistants et peuvent être toxiques (Bedoux et al. 2012). Le TCS est rapidement absorbé par le tractus gastro-intestinal et conjugué par l'uridine diphosphate-glucuronyltransférase hépatique dans ses formes glucuronidées (Sandborgh-Englund et al. 2006). Il a été trouvé dans le plasma humain (Allmyr et al. 2006), l'urine (Lu et al. 2016, Provencher et al. 2014) et le lait maternel (Adolfsson-Erici et al. 2002; Allmyr et al. 2006).

2.4.2 Les effets du BPA et du TCS sur la santé

Bien qu'il n'ait pas de cohérence dans la littérature sur les effets du BPA sur la santé dans la population générale (Bae and Hong 2015; Shiue 2014a, Wang et al., 2015), le BPA a été associé à des conditions humaines telles que les maladies cardiovasculaires, l'obésité, le diabète de type 2, les maladies respiratoires, les maladies rénales chroniques, les anomalies congénitales et les troubles du développement, les troubles du comportement, le cancer et les maladies auto-immunes (Rezg et al. 2014). Dans leur revue de la littérature, Han and Hong (2016) ont émis l'hypothèse d'un possible lien entre le BPA et l'hypertension artérielle. En utilisant des données provenant de *US National Health and Nutritional Examination Survey* (NHANES), Shankar and Teppala (2012) ont montré dans cette étude transversale (n = 1380) une association positive entre le BPA et la TA élevée. Bae and Hong (2015) ont trouvé des résultats similaires avec leur essai croisé randomisé. Par contre, Bae et al. (2012) ont rapporté qu'une association que chez ceux (n = 258) n'ayant pas d'antécédent d'hypertension artérielle alors qu'Aekplakorn et al. (2015) dans une étude transversale (n = 2588) ont observé qu'une association positive chez les femmes, mais

pas chez les hommes. À l'inverse des autres auteurs, Wang et al. (2015) ont rapporté au sein de leur étude transversale (n = 3246) une relation négative entre le BPA et l'hypertension artérielle alors que d'autres ont montré une absence d'association (Shiue 2014a; Shiue and Hristova 2014).

Les études examinant la relation entre le BPA et l'hypertension artérielle chez la femme enceinte sont rares. Seulement deux études ont été identifiées (Cantonwine et al. 2016; Leclerc et al. 2014). Ces deux dernières sont des études cas-témoins avec une taille d'échantillon de 482 et 58, respectivement, et elles ont toutes deux trouvé une association positive entre la prééclampsie et le BPA mesuré dans l'urine (Cantonwine et al. 2016) ou dans le placenta (Leclerc et al. 2014).

Pour ce qui est du TCS, une seule étude l'a mis en lien avec les maladies cardiovasculaires. Cullinan et al. (2015) ont trouvé une association entre le TCS contenu dans les dentifrices et les niveaux faibles des biomarqueurs des maladies cardiovasculaires (lipoprotéines totales, lipoprotéines à haute densité et des lipoprotéines de faible densité). Cependant, il y a très peu de données disponibles sur son lien avec l'hypertension artérielle. Deux études seulement ont été trouvées examinant la rélation entre le TCS et la TA (Shiue 2014b; Shiue and Hristova 2014). Ces dernières sont des études tranversales effectuées dans la population générale et elles n'ont pas montré une association entre le TCS et la TA. Aucune étude n'a été trouvée chez la femme enceinte examinant la relation entre le TCS et l'hypertension arterielle ou la TA. Toutes les études portant sur le BPA et le TCS sont aussi décrites dans le tableau 38 dans l'annexe 2.

3. Résumé sur l'état des connaissances

En résumé, nous constatons à travers cette revue de littérature que les résultats des études sont divergents concernant l'impact potentiel des métaux ou d'agents chimiques comme le BPA ou le TCS, et qu'il n'existe que peu d'informations disponibles sur :

- La relation entre les niveaux du Hg, de l'As ou du Cd, et le risque de développer les désordres hypertensifs de la grossesse
- La relation entre l'exposition aux amalgames dentaires (présence ou remplacement) et le risque de développer les désordres hypertensifs de la grossesse
- La relation entre l'exposition au BPA ou le TCS et le risque de développer les désordres hypertensifs de la grossesse.

Nous proposons donc dans cette thèse d'analyser l'association entre les métaux, les amalgames dentaires, le BPA ou le TCS et les désordres hypertensifs de la grossesse dans l'étude prospective de cohorte des femmes enceintes de MIREC.

Chapitre 3 - Hypothèses et objectifs

Hypothèses

a. Les niveaux maternels du Pb, du Hg, du Cd, du Mn et de l'As sont associés aux désordres hypertensifs de la grossesse.
b. Les amalgames dentaires (source de Hg) et leur remplacement pendant la grossesse sont associés aux désordres hypertensifs de la grossesse.
c. Une association positive existe entre l'exposition aux phénoles (BPA et TCS) et les désordres hypertensifs de la grossesse.

Objectif principal

L'objectif principal de cette thèse est d'évaluer l'association entre les niveaux des métaux (As, Pb, Cd, Hg ou Mn), du BPA, du TCS ou exposition aux amalgames dentaires (source de Hg) et les désordres hypertensifs de la grossesse.

Objectifs spécifiques

Les objectifs spécifiques sont :

3.1 Objectif #1 (article 1)

Analyser l'association entre les niveaux maternels d'As, de Pb, de Cd, de Hg ou de Mn et les désordres hypertensifs de la grossesse (l'hypertension gestationnelle globale, l'hypertension gestationnelle et la prééclampsie).

3.2 Objectif #2 (article 2)

Analyser l'association entre les expositions maternelles aux amalgames dentaires (présence et remplacement) et l'hypertension gestationnelle globale.

3.3 Objectif #3 (article 3)

Analyser l'association entre les niveaux maternels de BPA ou de TCS et l'hypertension gestationnelle incluant la prééclampsie.

Chapitre 4 - Méthodologie

4.1 Conception de l'étude et population

L'étude MIREC est la première grande étude de cohorte canadienne qui vise à évaluer l'impact des produits chimiques présents dans l'environnement sur la santé des femmes enceintes et de leur enfant. C'est une étude de cohorte prospective réalisée auprès de 2001 femmes enceintes recrutées dans 10 villes canadiennes (Vancouver, Edmonton, Winnipeg, Sudbury, Ottawa, Kingston, Hamilton, Toronto, Montréal et Halifax). L'étude s'est étendue sur une période de 4 ans pour le recrutement (2008-2011). Parmi les 2001 femmes enceintes recrutées, 18 se sont retirées de l'étude en cours de suivi et 74 ont été exclues suite à une fausse couche ou une mortinaissance. La taille de l'échantillon finale de l'étude est de 1909 participantes. Tous les détails sur la population de MIREC sont donnés à l'annexe 4a. Le protocole original de l'étude MIREC est en annexe 4b. Les femmes enceintes participantes à l'étude ont été choisies selon les critères d'inclusion et d'exclusion présentés aux sections suivantes.

4.1.1 Les critères d'inclusion

Les critères d'inclusion étaient : la capacité de consentir et de communiquer en anglais ou en français, être âgée de 18 ans ou plus, avoir une grossesse au premier trimestre (6-13 semaines), planifier d'accoucher dans un hôpital qui participait à l'étude.

4.1.2 Les critères d'exclusion

Les femmes avec l'une des histoires médicales suivantes ont été exclues de l'étude: 1) les femmes qui ont connu durant la grossesse en cours des anomalies fœtales (par exemple la môle hydatiforme), des anomalies chromosomiques fœtales ou des malformations majeures, 2) les

femmes qui ont des antécédents de complications médicales comme l'insuffisance rénale avec une fonction rénale altérée, l'épilepsie, le lupus érythémateux et la sclérodermie, les maladies actives et chroniques du foie (hépatite), les maladies cardiaques, la maladie pulmonaire grave, le cancer, troubles hématologiques (patientes souffrant d'anémie ou thrombophilies ont été incluses), 3) les femmes avec menace d'avortement spontané, 4) les femmes avec antécédents d'hémorragie au premier trimestre (celles ayant la viabilité du fœtus documentée au moment du recrutement ont été incluses), 5) femmes avec utilisation de drogues illicites.

4.1.3 Les considérations éthiques

L'étude MIREC a été approuvée par le Comité d'éthique de la recherche de Santé Canada et le comité d'éthique de la recherche sur les humains du CHU de Sainte-Justine de Montréal, Québec (Canada) (centre de coordination du projet de recherche) ainsi que par les centres affiliés à l'étude MIREC. La participation des patientes était volontaire avec signature du consentement éclairé et le droit de se retirer de l'étude en tout temps sans pénalité ou perte des prestations auxquelles elles avaient droit. La confidentialité des informations est maintenue à travers un système de codification numérique des questionnaires et des spécimens biologiques. Les noms et les adresses des participantes ne sont accessibles qu'aux chercheurs du site et de leur personnel de recherche.

4.2 La collecte des données

4.2.1 Les questionnaires et le dossier médical

Les questionnaires prénataux administrés au premier, deuxième et troisième trimestre et le dossier médical ont permis de recueillir les informations sur les potentielles sources d'exposition aux métaux, BPA et TCS, le statut de tabagisme et les variables importantes telles que les

facteurs sociodémographiques et les facteurs confondants potentiels tels que la parité, la grossesse gémellaire et le diabète gestationnel. De plus, un questionnaire de fréquence alimentaire a été administré durant la visite 2 pour obtenir des données sur la consommation de poissons, de calcium et d'autres informations diététiques (voir annexe 4c).

Pour les amalgames dentaires, l'information a été obtenue au moyen d'un questionnaire administré aux visites 1 et 3. On a demandé aux femmes à la première et à la troisième visite : « actuellement, combien d'amalgames dentaires en mercure-argent avez-vous? » Elles ont également été interrogées lors des visites 1 et 3 : « Au cours des 12 derniers mois, avez-vous remplacé des obturations dentaires en mercure-argent (aussi appelées amalgame) ? » Et « Depuis la première visite, avez-vous remplacé des obturations dentaires en mercure-argent (aussi appelées amalgame) ? » (voir annexe 4c). Les caractéristiques des amalgames dentaires n'ont pas été spécifiquement analysées.

4.2.2 Les prélèvements de sang et d'urine

Des prélèvements sanguins et urinaires ont été effectués au cours de la grossesse pour les dosages des métaux, du BPA et du TCS. Des échantillons maternels ont été prélevés au cours du premier et du troisième trimestre pour le dosage des métaux sanguins (As, Pb, Cd, Hg et Mn) et au premier trimestre pour les mesures urinaires de l'arsenic (As (III), As (V), arsénobétaïne, MMA et DMA), du BPA, du TCS et de la densité spécifique (voir annexe 4e).

Le sang maternel a été recueilli au 1er et au 3^{ième} trimestre dans des tubes K2EDTA de 6 ml et a été aliquoté dans des tubes Sarstedt® de 5 ml (Sarstedt AG & Company, Nümbrecht, Allemagne) et congelé à -20°C. Des échantillons ont été expédiés à l'état congelé au Centre de Toxicologie du Québec, à l'Institut National de Santé Publique du Québec (INSPQ), Québec QC,

Canada pour analyses. Les échantillons de sang maternel (500 µl) ont été dilués 20 fois dans un diluant contenant l'hydroxyde d'ammonium à 0.5% (volume/volume) et 0.1% (volume/volume) d'éthoxylate d'octylphénol. Les courbes d'étalonnage externes ont été préparées en diluant 20 fois, le volume correspondant de sang humain (à partir de volontaires sains) avec un diluant et ensuite en utilisant différents volumes de solution standard à plusieurs éléments de 1mg/l (SCP Science, PlasmaCal ICP-MS Verification Standard 1 ; 5% HNO3, n ° 141-110-011). Les standards internes pour la courbe d'étalonnage et l'analyse des échantillons de sang étaient Yttrium (^{89}Y) (pour ^{55}Mn), Rhodium (^{103}Rh) (pour ^{114}Cd), Terbium (^{159}Tb) (pour ^{208}Pb), Platine (^{195}Pt) (pour ^{202}Hg) et ^{89}Y (pour ^{75}As). La qualité interne a été assurée par la mise en œuvre de matériaux de référence validés (QMEQAS08B05, QMEQAS08B08, QM-B-Q1108 et QM-B-Q1201 : sang humain du système de comparaison inter-laboratoire du plan d'évaluation de la qualité externe du Québec (QMEQAS) après étalonnage, après chaque 10^{ième} échantillon ainsi qu'à la fin de chaque séquence analytique. Les échantillons d'urine du premier trimestre ont été recueillis dans des récipients Nalgene® de 125 ml (Thermo-Fisher Scientific Inc., Rochester NY, États-Unis), inclus dans des récipients Nalgene® de 30 ml, congelés à -20°C dans les 2 h de collecte et expédiés en glace sèche au centre de coordination MIREC à Montréal où ils ont été stockés à -30°C. L'urine (100 µl) a été diluée 1/10 avec un solvant d'élution et analysée sans étapes de préparation d'échantillon antérieures (Arbuckle et al. 2016; Ettinger et al. 2017).

Les mesures sanguines des métaux ont été effectuées par une méthode de spectrométrie de masse par plasma à couplage inductif à un seul quadripôle (ICP-MS) Elan DRC-II (Perkin Elmer, Norwalk CT, USA). La Chromatographie liquide haute performance (HPLC) couplée à l'ICP-MS (Varian 820-MS, Varian Inc., Palo Alto CA, États-Unis) a été utilisée pour les mesures urinaires

de l'As et la chromatographie liquide sensible couplée à la méthode de spectrométrie de masse en tandem (LC-MS/MS) pour le BPA et le TCS. Pour contrôler la dilution urinaire, la densité spécifique a été mesurée par réfractométrie (UG-1, Atago # 3461, Atago U.S.A. Inc., Bellevue WA, USA). Toutes les analyses ont été réalisées auprès du Centre de toxicologie du Québec (INSPQ), Québec, Canada.

Dans cette étude, une valeur équivalente à la moitié de LDD a été attribuée à des concentrations sanguines et urinaires inférieures à la LDD. L'As (III), l'As (V), le MMA et le DMA ont été regroupés dans As inorganique. Tous les As urinaires ont été regroupés dans As total. Le BPA libre, glucuronide, disulfure et monosulfure ont été groupés dans BPA. TCS libre, glucuronide et sulfate ont été groupés dans TCS. Les détails des valeurs de référence ou de LDD des métaux pour l'étude MIREC et pour le Québec (LeBlanc et al. 2003) sont résumés dans le tableau 10.

Tableau 10. Valeurs de référence ou de limite de détection (LDD) des métaux pour l'étude MIREC et le Québec

Métaux	Niveau à signaler (MIREC)	Niveau à signaler (Québec) ou LDD	LDD (MIREC)
Pb	4 µg /dl (0.19µmol/l)	10 µg/dl (0.5 µmol/l)	0.1036 µg /dl (0.001 µmol/l)
Hg	8 µg /l (40 nmol/l)	12 µg/l (60 nmol/l)	0.1204 µg /l (0.6 nmol/l)
Cd	5.1 µg /l (45 nmol/l)	5.1 µg/l (45 nmol/l)	0.0454 µg /l (0.4 nmol/l)
Mn	n/a	365 nmol/l (20.06 µg/l)	0.54945 µg /l (10 nmol/l)
As total	n/a	3 nmol/l (0.23 µg/l)	0.2247 µg /l (3 nmol/l)
As (III)	n/a	0.06 µmol/l (4.49 µg/l)	0.75 µg As/l (0.01 µmol/l)
As (V)	n/a	0.08 µmol/l (5.99 µg/l)	0.75 µg As/l (0.01 µmol/l)
Arsénobétaïne	n/a	0.02 µmol/l (1.50 µg/l)	0.75 µg As/l (0.01 µmol/l)
MMA	n/a	0.04 µmol/l (3 µg/l)	0.75 µg As/l (0.01 µmol/l)
DMA	n/a	0.02 µmol/l (1.50 µg/l)	0.75 µg As/l (0.01 µmol/l)

Source: Tableau inspiré des données du projet MIREC et des valeurs de référence d'INSPQ (LeBlanc et al. 2003).
MMA = acide monométhylarsonique. DMA = acide diméthylarsinique.

Toutes les informations sur les analyses sanguines et urinaires des métaux, du BPA et TCS sont détaillées aux chapitres 5, 6 et 7. Aussi tous les détails sur la méthodologie de l'étude MIREC sont donnés en annexe 4.

4.3 Les principales variables à l'étude

4.3.1 La variable dépendante

Cette variable dépendante a été considérée comme une variable continue ou catégorielle selon le contexte spécifique aux 3 objectifs. La présence ou l'absence des désordres hypertensifs de la grossesse a été documentée sur la base de deux indicateurs: 1) deux mesures de la TA lors de chaque visite des participantes au cours de leur suivi de grossesse et 2) les informations

provenant du dossier hospitalier lors de l'admission pour l'accouchement. Les mesures de la TAS et TAD maternelle ont été effectuées lors de chaque visite clinique par le personnel médical à l'aide d'un sphygmomanomètre. La TA a été prise chez la patiente en position assise, le bras au repos sur un bureau au niveau du cœur. La TAD était mesurée à la phase V de Korotkoff (disparition) et IV (étouffement) seulement utilisée quand la phase V était absente. Les données concernant les mesures de la TA effectuées lors de l'hospitalisation pour l'accouchement ont été relevées du dossier médical.

Dans ce travail, les critères diagnostiques pour les désordres hypertensifs de la grossesse sont basés sur les lignes directrices de la SOGC (Magee et al. 2014). Selon cette directive, le diagnostique d'hypertension artérielle est basé sur la moyenne d'au moins deux mesures de la TAS ≥140 mmHg et/ou de TAD ≥90 mmHg, prises à au moins une minute d'intervalle dans l'étude MIREC. L'apparition de l'hypertension artérielle à ≥20 semaines de gestation avec ou sans protéinurie ou complications maternelles définit l'hypertension gestationnelle globale. La présence de la protéinurie (protéinurie de 24h ≥ 300 mg/24h ou ≥ 0.3 g/24-h ou ≥ 30 mg/mmol de créatinine urinaire, ≥ 1+ à la bandelette urinaire) ou de complications maternelles définit la prééclampsie tandis que leur absence définit l'hypertension gestationnelle (Magee et al. 2014). L'hypertension gestationnelle et la prééclampsie sont traitées comme les sous-catégories de l'hypertension gestationnelle globale. L'hypertension chronique n'a pas été incluse dans la classification de ce travail car seuls les désordres hypertensifs en lien exclusivement avec la grossesse sont l'objet de cette thèse de doctorat. Les proportions pour les catégories des désordres hypertensifs de la grossesse sont les suivantes : 1630 (85.4%) pour les femmes normotendues, 187 (9.8%) pour l'hypertension gestationnelle globale (ce qui inclut 129 (6.8%) pour

l'hypertension gestationnelle et 58 (3%) pour la prééclampsie). Les femmes avec l'hypertension chronique (n = 58 (3%)) ou sans données ou informations pour l'hypertension artérielle (n= 34 (1.8%)) ont été traitées comme des données manquantes et n'ont pas été incluses dans la définition des désordres hypertensifs de la grossesse.

La TA moyenne (TAM) a été déterminée pour la visite 1 (6 à 13 semaines), la visite 2 (16 à 21 semaines), la visite 3 (32 à 34 semaines) et la visite 4 (après l'admission pour l'accouchement). Cette TAM a été calculée selon la formule suivante : (TAS + 2 TAD)/3. Contrairement aux désordres hypertensifs de la grossesse, la TAM a tenu compte des mesures de la TA du premier trimestre de la grossesse. L'âge gestationnel en semaines a été défini sur la base de la date de la dernière menstruation et/ou l'échographie précoce. La date de la dernière menstruation était principalement utilisée. L'échographie n'était utilisée que s'il y avait une différence de plus de 5 jours entre l'âge gestationnel calculé avec la dernière menstruation et celui de l'échographie précoce du premier trimestre de la grossesse.

4.3.2 Les variables indépendantes

Les niveaux sanguins de Pb, de Hg, de Cd, d'As et de Mn et les niveaux d'As urinaire (As (III), As (V), arsénobétaïne, MMA et DMA), ainsi que les niveaux urinaires de BPA et de TCS sont des variables indépendantes continues. Le nombre d'amalgames dentaires (0, 1-4 et ≥5) et leur remplacement (non et oui) sont des variables indépendantes catégorielles.

4.3.3 Les variables de confusion potentielles

Les données sur les covariables potentielles proviennent de questionnaires prénataux administrés au premier, deuxième et troisième trimestre ainsi qu'aux examens des dossiers médicaux.

- Tabagisme maternel : Il a été mesuré comme une covariable catégorielle et les données ont été recueillies à partir des questionnaires au premier et au troisième trimestre et du dossier médical. Cette covariable a été catégorisée en 4 catégories pour le premier objectif (jamais, cessé de fumer avant la grossesse, cessé de fumer pendant la grossesse, fume à l'heure actuelle) et en 2 catégories (non, oui) pour les autres objectifs.

- Indice de masse corporelle (IMC) : Covariable continue pour le deuxième objectif et catégorielle (poids insuffisant, poids normal, surpoids, obésité) pour l'objectif 1 et 3. Les mesures du poids ont été réalisées à chaque trimestre et recueillies à partir du dossier médical lors de l'admission pour l'accouchement. Pour la taille, la mesure a été faite au 1er trimestre consignée dans le dossier médical. L'IMC (kg/m^2) = poids (kg)/taille (m^2).

- **Parité** : Covariable catégorielle (multipare/nullipare). Les données ont été recueillies à partir des questionnaires du premier trimestre.

- **Âge maternel en années** : Covariable continue pour les deux premiers objectifs et catégorielle (18-31, 32-35, 36-49) pour le troisième. Les données ont été recueillies à partir des questionnaires du premier trimestre et du dossier medical.

- **Ethnicité** : Covariable catégorielle (caucasienne, non-caucasienne). Les données ont été recueillies à partir des questionnaires du premier trimestre.

- **Revenu familial** : Covariable catégorielle (≤15,000, 16,000-35,000, 36,000-45,000, 46,000-55,000, 56,000-65,000, 66,000-75,000, 76,000-90,000, > 90,000 $ CAN pour le premier objectif et < 65,000, 65,000 – 90,000, > 90,000 $ CAN pour les autres). Les données ont été recueillies à partir des questionnaires du premier trimestre.

- C**onsommation d'alcool pendant les 3 mois précédant la visite 1** : Covariable binaire (non, oui). Les données ont été recueillies à partir des questionnaires du premier trimestre.

- **Consommation de poisson** : Covariable continue basée sur la quantité totale par jour calculée à partir du questionnaire sur la fréquence des aliments par Morisset et al. (2016). Les données ont été recueillies lors de la visite du deuxième trimestre.

- **Niveau de scolarité** : Covariable en 3 catégories (études supérieures, université et inférieur à l'université) pour le premier et troisième objectif, en 4 catégories pour le deuxième (diplôme d'études supérieures, diplôme de premier cycle, collège et inférieur au collège). Les données ont été recueillies à partir des questionnaires du premier trimestre.

- **Gain de poids durant la grossesse** : Covariable catégorielle seulement pour le 3^{e} objectif (<12.40, 12.40-17.24, >17.24 kg). C'est la difference entre le poids mesuré au premier trimestre au moment de la première visite et le poids mesuré à la dernière visite avant l'accouchement, recueilli à partir du dossier médical.

- **Consommation de café** : Covariable catégorielle (non, oui). Les données ont été recueillies à partir des questionnaires du premier et troisième trimestre et par ceux de la fréquence alimentaire administrés pendant la 2^{e} visite.

- **Grossesse multiple** : Covariable catégorielle (non, oui). Les données ont été recueillies à partir des questionnaires du premier trimestre.

- **Antécédent de maladie auto-immune** : Covariable catégorielle (non, oui). Les données ont été recueillies à partir des questionnaires du premier trimestre.

Le diabète gestationnel est une covariable binaire (non/oui) et les données ont été recueillies à partir des questionnaires du premier, deuxième et troisième trimestre et du dossier

médical. Cette covariable a été exclue des analyses statistiques pour avoir des données manquantes trop importantes (34.5%). L'âge maternel, la parité, l'ethnicité, le niveau de scolarité, l'IMC, le revenu familial, le tabagisme maternel, consommation d'alcool pendant les 3 mois précédant la visite 1, la consommation de poisson, le gain de poids durant la grossesse et la densité spécifique sont analysés comme des variables de confusion potentielles pour le premier objectif. Pour le deuxième objectif, on a tenu compte de l'âge maternel, la parité, l'ethnicité, l'IMC, le revenu familial, le tabagisme maternel, le niveau de scolarité, le gain de poids durant la grossesse, la consommation de café, grossesse multiple, maladie auto-immune et la consommation de poisson. De même, l'âge maternel, la parité, l'ethnicité, le niveau de scolarité, l'IMC, le revenu familial, le gain de poids durant la grossesse et le tabagisme maternel ont été analysés pour le troisième objectif (voir annexe 5).

Parmi ces variables de confusion potentielles, l'âge maternel a été choisi *a priori* sur la base de la littérature comme facteur de confusion pour les 3 objectifs tandis que pour l'objectif 3, en plus de l'âge maternel, la scolarité, le revenu familial et le tabagisme maternel ont été choisis sur la base de la littérature. Les facteurs de confusion pour le premier objectif sont l'âge maternel, l'ethnicité, la scolarité, l'IMC, le revenu familial, le tabagisme maternel, la consommation de poisson, le gain de poids durant la grossesse et la densité spécifique. Pour le deuxième objectif, l'âge maternel, l'IMC, la consommation de poisson ou de café, le gain de poids durant la grossesse sont les facteurs de confusion. Pour le troisième objectif, l'âge maternel, la scolarité, le revenu familial, le tabagisme maternel, la densité spécifique et le gain de poids durant la grossesse sont les facteurs de confusion. Les relations entre les différentes variables (exposition, issue, variables de confusion et d'interaction) sont décrites dans des cadres conceptuels

correspondant à chaque objectif (voir annexe 3). La liste des variables évaluées dans l'étude MIREC est présentée à l'annexe 4d.

4.4 Analyse statistique

L'analyse statistique est réalisée selon les objectifs. Des analyses descriptives sont effectuées pour dresser le portrait des caractéristiques principales des participantes selon la distribution des catégories des désordres hypertensifs de la grossesse présentes dans la population d'étude et les niveaux d'expositions aux métaux, au BPA, au TCS et aux amalgames dentaires. La densité spécifique est une variable continue qui a été utilisée comme une covariable pour tenir compte de l'effet de la dilution urinaire pour les mesures urinaires de l'As, du BPA et du TCS. La mesure a été effectuée sur l'échantillon de l'urine prélevé à la visite du premier trimestre.

Pour cette analyse, nous avons utilisé l'ensembre de la cohorte avec une taille d'échantillons de 1909 femmes enceintes. L'hypertension chronique a été traitée comme des données manquantes dans la définition des désordres hypertensifs de la grossesse. Dans celle de la TAM, les mesures de la TA au premier trimestre ont été aussi prises en compte contrairement à la définition des désordres hypertensifs de la grossesse.

4.4.1 Pour le premier objectif

Objectif 1 (article 1) : Analyser l'association entre les niveaux maternels d'As, de Pb, de Cd, de Hg ou de Mn et les désordres hypertensifs de la grossesse (l'hypertension gestationnelle globale, l'hypertension gestationnelle et la prééclampsie).

Des statistiques descriptives ont été initialement effectuées pour analyser les caractéristiques des participantes selon les désordres hypertensifs de la grossesse en utilisant les tests T de Student, Mann-Whitney, Kruskal-Wallis ou Chi-carré. Les moyennes géométriques

(MG) (avec 95% IC) et les distributions catégorielles des métaux ont été déterminées pour chaque catégorie des désordres hypertensifs de la grossesse (normotensive, hypertension gestationnelle globale, hypertension gestationnelle et prééclampsie). Les niveaux des métaux ont été comparés en utilisant les tests de Mann-Whitney et Kruskal-Wallis et les tertiles ont été comparés en utilisant le test du Chi-carré. Des corrélations de Spearman ont également été effectuées pour tester la corrélation entre les mesures des métaux dans le sang au premier et troisième trimestre et pour tester la corrélation entre l'As sanguin et urinaire.

Nous avons utilisé plusieurs approches analytiques pour étudier les relations entre les métaux et le risque de désordres hypertensifs de la grossesse. Des modèles de régression logistique ont été effectués pour tester la relation entre les métaux et l'hypertension gestationnelle globale par rapport aux femmes normotendues, tandis que les modèles de régression multinomiale ont été réalisés avec les désordres hypertensifs de la grossesse définis selon trois groupes (femmes normotendues, hypertension gestationnelle et prééclampsie). Tous les métaux ont été testés individuellement dans chaque modèle en tant que variables continues et catégorielles (tertiles) pour considérer différentes alternatives pour modéliser l'exposition. Les résultats sont regroupés dans les tableaux selon le type d'échantillon recueilli (sang par rapport à l'urine) et le trimestre de collecte des échantillons. La valeur $p < 0.05$ a été considérée comme indiquant une signification statistique.

Afin de contrôler le biais de confusion potentiel, les covariables ont été choisies soit *a priori* sur la base de la littérature (âge maternel), soit selon la méthode de changement dans le risque relatif. Toutes les covariables ont été examinées individuellement et celles qui ont changé de +/- 10% l'odds ratio (OR) pour la relation entre les métaux et les désordres hypertensifs de la

grossesse ont été considérées comme des facteurs de confusion et ont été retenues dans chaque modèle multivarié correspondant. Nous avons également testé l'interaction selon le sexe du bébé. Nous n'avons pas été en mesure de tester l'interaction selon le statut de tabagisme maternel en raison du petit nombre de fumeuses dans notre étude. Les résultats ont été stratifiés si le terme d'interaction était significatif à $p < 0.05$. Nous avons aussi effectué 2 analyses de sensibilité : 1) avec la consommation de poisson dans le modèle de mercure et 2) en incluyant tous les métaux sanguins dans le même modèle selon le trimestre de mesure.

Dans un ensemble d'analyses distinctes, nous avons exploré la relation entre les mesures concurrentes des métaux et de TAM en utilisant des modèles d'équations d'estimation généralisées linéaires (GEE), qui tiennent compte du regroupement au sein de chaque individu causé par la conception des mesures répétées. Les modèles ont incorporé un modèle de corrélation autorégressif de premier degré pour les événements répétés. La TAM a été estimée pour chaque femme à chaque visite (de chaque mesure individuelle recueillie dans la visite correspondante) et elle a été utilisée comme l'issue mesurée en continu dans les modèles de GEE linéaire. Les métaux ont été transformés en logarithme naturel (ln) pour se rapprocher d'une distribution normale. Les modèles de GEE linéaire ont été réalisés en utilisant des mesures simultanées des métaux et la TAM aux visites 1 et 3. Ce modèle a permis d'examiner l'impact direct de chaque métal prélevé au même moment que la mesure de TA. Étant donné que l'As urinaire n'a été mesuré qu'une fois lors de la première visite, nous avons utilisé une régression linéaire pour analyser son association avec la mesure de la TAM lors de la premiére visite.

Pour l'ensemble des analyses, les données manquantes (peu nombreuses) ont été considérées « *missing completely at random* (MCAR) ». Afin de permettre plus de puissance aux

modèles multivariés, les covariables des modèles ont été introduits en continue lorsque possible et les données manquantes ignorées ce qui a pu diminuer le nombre des femmes incluses dans les modèles multivariés. Comme les données manquantes n'étaient pas nombreuses et considérées MCAR, cela n'a pas eu d'impact important sur les résultats.

4.4.2 Pour le deuxième objectif

Objectif 2 (article 2) : Analyser l'association entre les expositions maternelles aux amalgames dentaires (présence et remplacement) et l'hypertension gestationnelle globale.

Les statistiques descriptives des caractéristiques maternelles ont été estimées selon le statut de l'amalgame dentaire (nombre d'amalgames dentaires déclarés au début ou au troisième trimestre, remplacement des amalgames dentaires au cours des 12 derniers mois signalés lors de la visite du premier trimestre et le remplacement pendant la grossesse lors de la visite du troisième trimestre. Les comparaisons pour les variables continues ont été menées avec le test T de Student, ou les tests Anova, Mann-Whitney ou Kruskal-Wallis et pour les variables catégorielles en utilisant les tests Chi-carré. Les médianes (intervalle interquartile (IQR)) et les MG du Hg ont été déterminées selon le statut de l'amalgame dentaire et ont été comparées à l'aide de Mann-Whitney ou de Kruskal-Wallis. Enfin, la corrélation de Spearman a été utilisée pour tester les corrélations entre le Hg sanguin, le statut de l'amalgame dentaire et la consommation de poisson.

Des modèles de régression logistique ont été effectués pour explorer l'association entre le statut de l'amalgame dentaire et l'hypertension gestationnelle globale (oui ou non). La présence d'amalgames dentaires a été évaluée selon leur nombre (classé comme 0, 1-4 et ≥ 5) et le moment où ils ont été signalés (visite du premier ou troisième trimestre). Nous avons également analysé

l'impact des remplacements des amalgames dentaires selon les réponses déclarées lors des visites du premier et du troisième trimestre, c'est-à-dire, remplacement dans les 12 mois précédant la visite du premier trimestre et remplacement pendant la grossesse (rapporté lors de la visite du troisième trimestre). Les rapports de cotes bruts et ajustés (OR (95% IC)) ont été utilisés. L'ajustement a été effectué pour les covariables choisies *a priori* sur la base de preuves de confusion potentielle documentées dans la littérature (âge maternel) ou empiriquement. Nous avons examiné d'autres covariables séparément pour un biais de confusion potentiel en utilisant la méthode de changement du risque relatif. Celles qui ont modifié les OR de l'association entre le statut d'amalgame dentaire et l'hypertension gestationnelle globale de +/- 10% ont été considérées comme un facteur de confusion et inclus dans le modèle multivarié. Les valeurs P de <0.05 ont été considérées comme indiquant une signification statistique. Nous avons fait deux analyses de sensibilité : 1) en considérant comme données manquantes les femmes qui ont signalé la présence d'amalgames dentaires ou pas au premier trimestre mais ayant des données manquantes au troisième trimestre et 2) en tenant compte de la consommation de poisson dans les modèles.

Nous avons également utilisé des GEE logistique pour explorer la relation entre le statut d'amalgame dentaire (variable répétée mesurée au premier et au troisième trimestre) et l'hypertension gestationnelle globale (variable binaire non répétée et mesurée après le premier trimestre à $\geq$20 semaines). Le modèle GEE logistique prend en compte le regroupement au sein de chaque individu causé par la conception des mesures répétées. Les modèles ont incorporé un modèle de corrélation autorégressif de premier degré pour les événements répétés.

Pour l'ensemble des analyses de l'objectif 2, les covariables des modèles ont aussi été introduites en continue lorsque possible afin de permettre plus de puissance aux modèles. Les données manquantes ignorées ont pu diminuer le nombre des femmes dans les modèles multivariés, mais comme elles étaient peu nombreuses et considérées MCAR, cela n'a pas eu d'impact important sur les résultats.

4.4.3 Pour le troisième objectif

Objectif 3 (article 3) : Analyser l'association entre les niveaux maternels du BPA ou du TCS et l'hypertension gestationnelle et la prééclampsie.

Des statistiques descriptives ont été effectuées pour analyser les caractéristiques des participantes en fonction des niveaux urinaires de BPA et de TCS en utilisant Mann-Whitney et Kruskal-Wallis. La médiane (intervalle interquartile (IQR)) et la distribution catégorielle du BPA et du TCS ont été déterminées en fonction du statut de l'hypertension (femmes normotendues, hypertension gestationnelle et prééclampsie). Les distributions des médianes (IQR) selon les tertiles ont été comparées à l'aide du test de Kruskal-Wallis et du Chi-carré, respectivement. La corrélation de Spearman a été utilisée pour tester la corrélation entre BPA et TCS.

Des modèles de régression multinomiale ont été effectués pour calculer les OR (95% IC) pour l'association entre le BPA ou le TCS et le statut d'hypertension. Le BPA et le TCS ont été testés individuellement dans chaque modèle en tant que variable continue et en tant que variable catégorielle (tertiles) afin de considérer différentes alternatives pour modéliser l'exposition. L'âge maternel, le tabagisme maternel, le revenu du ménage et l'éducation qui ont été identifiés dans l'étude MIREC par Arbuckle et al. (2015) en tant que prédicteurs pour le BPA et le TCS ont été considérés *a priori* comme des variables de confusion potentielles et ont été inclus dans des

modèles multivariés parce qu'ils sont également impliqués dans des désordres hypertensifs de la grossesse selon la littérature. En outre, toutes les autres covariables susmentionnées ont été examinées empiriquement comme des facteurs de confusion potentiels en utilisant la méthode "Change-in-estimations". Celles qui ont changé de +/- 10% de l'OR pour l'association entre le BPA ou le TCS et le statut de l'hypertension ont été considérées comme un facteur de confusion et inclus dans chaque modèle multivarié correspondant. La valeur P <0.05 a été considérée comme indiquant une signification statistique.

Pour l'ensemble des analyses de l'objectif 3, les covariables des modèles ont aussi été introduites en continue lorsque possible et les données manquantes ignorées ce qui a pu diminuer le nombre des femmes dans les modèles multivariés. Par contre, comme les données manquantes n'étaient pas nombreuses et considérées MCAR, cela n'a pas eu d'impact important sur les résultats.

Toutes les analyses statistiques ont été effectuées avec IBM SPSS Statistics version 22, SAS 9.4 pour Windows, StataSE 14 et R pour Windows 3.2.2. D'autres détails de la méthodologie, de l'analyse statistique et les résultats sont donnés dans les 3 articles présentés aux chapitres 5, 6 et 7.

4.5 Calcul de la puissance

Avec une taille d'échantillon de 1909 (1630 normotendues et 187 participantes avec l'hypertension gestationnelle globale), une puissance de 80% et un paramètre alpha de 0.05 ainsi qu'une proportion d'exposition d'environ 33%, nous étions en mesure de détecter un OR minimal de 1.576 si P1 est supposé plus grand que P0 ou de 0.603 si P1 est supposé plus petit que P0 (voir annexe 6).

4.6 Contribution globale de l'étudiante

Je suis arrivée à un moment où l'étude MIREC était déjà dans sa phase d'exécution. Je n'ai donc pas participé à la formulation du projet MIREC (l'identification et l'obtention du financement) ni à sa planification (la soumission au comité d'éthique, le plan de travail que ce soit scientifique ou logistique, les ressources humaines, matérielles, informationnelles et financières) ni aux phases initiales de son exécution (la mise en œuvre : la constitution de l'équipe, la collecte des données, le suivi, le contrôle, la saisie, la validation). J'ai participé à la phase finale d'exécution de l'étude MIREC (analyse et interprétation) pour l'exploitation des données et la publication d'articles scientifiques.

Dans ce travail, j'ai réalisé la revue de littérature qui traite des désordres hypertensifs de la grossesse et de leur relation avec l'exposition aux métaux, aux amalgames dentaires, au BPA et au TCS. J'ai aussi élaboré les objectifs et choisi le format par article de la thèse. J'ai également fait ressortir toutes les variables à l'étude et leurs caractéristiques, les forces et faiblesses de mon projet et sa pertinence en santé publique. À travers la revue de littérature, l'ensemble des questionnaires de l'étude MIREC et la disponibilité des résultats des analyses de laboratoire des échantillons de l'étude MIREC, j'ai sélectionné les variables à inclure dans la base de données de mon projet. Par la suite, j'ai extrait mes données de la base de données mère de l'étude MIREC pour produire ma propre base de données. J'ai élaboré mes propres variables et participé à leur création et au nettoyage de ma base de données. Par ailleurs, j'ai fait des recherches pour définir les valeurs de référence et/ou de détection de l'As sanguin et de la spécialisation de l'As urinaire. Malgré qu'ils soient mesurés dans l'étude MIREC, leurs valeurs de référence ne sont pas précisées dans le protocole. J'ai mené les mêmes recherches pour confirmer les valeurs de

références des autres métaux (Pb, Hg, Cd et Mn). Compte tenu des caractéristiques des principales variables utilisées, j'ai personnellement choisi le type d'analyse statistique et le logiciel à utiliser. J'ai choisis les titres des 3 articles de la thèse ainsi que les journaux de publication. J'ai fait toutes les revues de la littérature, les analyses et la rédaction des articles. J'ai activement participé aux analyses statistiques et aux interprétations des résultats. J'ai fait toute la rédaction de la thèse. Des informations supplémentaires me concernant sont décrites dans mon curriculum vitæ (voir l'annexe 7).

Chapitre 5 - Metals and hypertensive disorders of pregnancy: MIREC Study (Article 1)

Short title: metals and hypertensive disorders in pregnancy

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Statut de l'article : Le manuscrit est en correction à Santé Canada. Il doit être soumis au journal Environmental Health Perspectives.

Contribution des auteurs :

- **Louopou Rosalie Camara** a constitué la base de données, elle a fait toutes les analyses et la rédaction du manuscrit sous la supervision de Dre Helen Trottier et de Dr William D. Fraser.

- **Les coauteurs** : Dre Helen Trottier a participé aux analyses et à la rédaction du manuscrit. Dr William D. Fraser un l'un des chercheurs principaux de l'étude MIREC. Ils ont participé au design de l'étude, à sa conduite et aux analyses ainsi qu'à la rédaction du manuscrit.

Abstract

Background: Metals have been associated with hypertensive disorders of pregnancy (HDP) but data are scarce.

Objective: To assess the association between metals (arsenic, lead, cadmium, mercury, or manganese) and HDP.

Methods: Urinary arsenic was measured in the first trimester and blood metals in the first and third trimesters. Blood pressure was assessed by trimester in 2001 pregnant women recruited in 10 Canadian cities. Associations (adjusted odd ratios (aOR) and 95% Confidence intervals (CI)) between metals and HDP (gestational hypertension (GH) overall, GH and preeclampsia (PE)) were analyzed using logistic and multinomial regression models. Adjusted beta (aß) were also estimated using concurrent measures of metals and blood pressure to analyze the associationsusing linear generalized estimating equations

Results: First trimester blood manganese (> 9.89 versus <7.69 µg/l) was associated with lower risks of GH overall (aOR = 0.68; 95% CI: 0.46, 0.99) and PE (aOR=0.48; 95% CI: 0.23, 0.98), especially in women delivering males babies. When measured concurrently, increasing blood manganese was associated with higher blood pressure (aß=1.52; 95% CI: 0.80-2.24). Third trimester blood mercury (> 0.82 versus <0.36 µg/l) was also associated with a lower risk of GH overall (aOR = 0.62; 95% CI: 0.39, 0.98). First trimester blood arsenic (> 0.97 versus 0.60 µg/l) was associated with an elevated risk of PE, adjusted for the other metals (aOR = 2.75; 95% CI 1.13, 6.73). No significant associations were observed for blood lead, cadmium or urinary arsenic.

Conclusion: Manganese and mercury were associated with lower odds of GH overall and/or PE whereas blood arsenic was associated with an increased risk of PE. Further studies are required to replicate these results in similar populations.

Introduction

Manganese (Mn) is an essential element required for growth, development, and maintenance of health (Avila et al. 2013). It plays a leading role in the formation of enzymes, in particular pyruvate carboxylase, glutamine synthetase and superoxide dismutase (Avila et al. 2013; Wood 2009) which are respectively important in glucose metabolism, expressed in astrocytes (Avila et al. 2013) and antioxidant enzymes that protect cells from damage due to free radicals (Candas and Li 2014). Although an essential element, Mn toxicity can also result from high (Avila et al. 2013; Vigeh et al. 2013) as well as low levels of exposure (Avila et al. 2013; Sarwar et al. 2013). Arsenic (As), lead (Pb), cadmium (Cd) and mercury (Hg) have no known physiological function (Jaishankar et al. 2014). Their toxicity depends on the dose, route of exposure, chemical species, age, gender, genetics, and nutritional status of exposed individuals (Tchounwou et al. 2012).

Human exposure to these metals has increased due to the expansion of their use in several applications (Tchounwou et al. 2012). Although, food is the primary source of exposure to Mn, it can also be found in drinking water, air (especially occupational exposures), and soil (Health Canada 2016). Food is a major source of cadmium (Cd), arsenic (As) and mercury (Hg) followed by cigarette smoke for Cd, drinking water for As (Järup 2003) and dental amalgam for Hg (Health Canada 2004; Järup 2003). Lead (Pb) can be present in jewellery, toys, cosmetics, contaminated food, dust, water (WHO 2016) and air (Järup 2003).

Exposure to these metals may induce low nitric oxide levels, endothelial dysfunction and increased oxidative stress (Jomova et al. 2011; Lemos et al. 2012; Cordova et al. 2013; Vaziri 2008) or may impact on the functioning of matrix metalloproteinases (MMPs) (Au et al. 2016; Lacorte et al. 2015). These mechanisms have been associated with gestational hypertension

without preeclampsia (GH) (Tayebjee et al. 2005; Kennedy et al. 2012) or preeclampsia (PE) (Chen and Khalil 2017; Powe et al. 2011). Hypertension in pregnancy, which includes GH and PE (Magee et al. 2014), contributes to maternal morbidity (Nakimuli et al. 2016), mortality (Lo et al. 2013) and represents 3.6% to 9.1% of pregnancies in developed countries (Roberts et al. 2011). Approximately 50% of women with GH go on to develop PE (Leeman et al. 2016) which affects 2-8% of pregnancies (Duley 2009) and which is a major source of perinatal morbidity and mortality (Leeman et al. 2016).

Epidemiological studies have observed an association between these metals and hypertensive disorders of pregnancy (HDP). Depending on the form in which it is measured, an association has been reported between As and blood pressure among pregnant women (Farzan et al. 2015). Methylated forms in urine (monomethylarsonic acid (MMA) and dimethylarsinic acid (DMA) is generally excreted) (Drobna et al. 2009) and are thought to be less toxic (Jomova et al. 2011). The natural organic form, arsenobetaine, may have minimal toxic effects (Jomova et al. 2011; Suzuki et al. 2002). Studies have reported a relationship between Pb and both PE (Ikechukwu et al. 2012; Motawei et al. 2013) and GH (Yazbeck et al. 2009). Cd exposure has been linked to GH, especially among smokers (Kosanovic and Jokanovic 2007), although this finding is not consistent, as smoking (which is a source of metals (OMS 2012)), has also been associated with a reduced risk of PE (Wikström and Stephansson 2010).

Associations between Hg and GH have been reported in both occupational (Pan et al. 2007) and non-occupational settings (Wells et al. 2017). But the adverse effect of Hg may be antagonized by fish containing omega-3 fatty (Houston 2011) due to their antioxidative properties (Dasilva et al. 2017). Some studies have shown a relationship between Mn and GH

(Vigeh et al. 2013) or PE (Sarwar et al. 2013). Finally, male fetus has been associated with PE (Myers et al. 2015) and it is possible that the relationship between metals exposure (Mn, Pb, Cd, Hg) and hypertension may be modified by the fetal sex (Bushnik et al. 2014; Lee et al. 2015; Nielsen et al. 2012) and maternal smoking status (Kosanovic and Jokanovic 2007).

Few cohort studies have analyzed the association between metal exposures and HDP and there is inconsistency in the literature (Maduray et al. 2017; Mordukhovich et al. 2012; Mozaffarian et al. 2012; Yazbeck et al. 2009). Studies of Hg, As and Cd are especially scarce. One of the main objectives of the MIREC Study (Maternal-Infant Research on Environmental Chemicals) was to investigate whether exposure to metals during pregnancy was associated with higher risks of HDP.

Methods

Study design and population

The MIREC Study is a prospective cohort study of 2001 pregnant women recruited from 10 Canadian cities (Vancouver, Edmonton, Winnipeg, Sudbury, Ottawa, Kingston, Hamilton, Toronto, Montreal, and Halifax). This study took place over a 4-year recruitment period (2008-2011). Women were eligible if they were in the first trimester of pregnancy without a history of significant medical complications, aged ≥18 years, and able to communicate in French or in English. Further details on the MIREC study population have been published previously (Arbuckle et al. 2013). After beginning the study, 18 women subsequently withdrew and asked that their data be destroyed, and 74 women were excluded because of miscarriage or stillbirth (including 3 cases of chronic hypertension and 1 case of preeclampsia), leaving a final sample size for this analysis of 1909 participants. The study was approved by Health Canada's Research

Ethics Board and the Research Ethics Committee of Sainte-Justine University Hospital in Montreal, Quebec (Canada) as well as in all MIREC affiliated recruitment centers. All participants signed consent forms.

During each trimester, women participated in a clinic visit where they provided biospecimens, had physical measurements taken and completed questionnaires on sociodemographic and exposure characteristics. Clinical data were also abstracted from the medical charts at each visit.

Blood and urine metals

Maternal whole blood from the first and third trimesters of pregnancy was analyzed for As, Pb, Cd, Hg, and Mn and maternal urines from the first trimester were analyzed for arsenite, arsenate, arsenobetaine, MMA, DMA and specific gravity (Arbuckle et al. 2016; Ettinger et al. 2017). The blood measurements were performed by a single-quadrupole inductively coupled plasma mass spectrometry (ICP-MS) Elan DRC-II system (Perkin Elmer, Norwalk CT, USA) method and high performance liquid chromatography coupled with ICP-MS (Varian 820-MS, Varian Inc., Palo Alto CA, USA) was used for urinary speciated As measurements. The specific gravity was measured by refractometry (UG-1, Atago # 3461, Atago U.S.A. Inc., Bellevue WA, USA). All laboratory analyses were performed by the Centre de Toxicologie du Québec, Institut National de Santé Publique du Québec (INSPQ), Quebec, Canada.

Limits of detection (LOD) were: 0.2247 µg/l (3 nmol/l) for blood As, 0.1036 µg/dl (0.001 µmol/l) for Pb, 0.0454 µg/l (0.4 nmol/l) for Cd, 0.1204 µg/l (0.6 nmol/l) for Hg and 0.54945 µg/l (10 nmol/l) for Mn. For urinary speciated As, the limit of detection was 0.75 µg As/l (0.01 µmol As/l). In this study, a value equivalent to the half of LOD was attributed to blood and urine

concentrations below the LOD. In terms of blood concentrations, no metal had more than 12% below the LOD. For urinary measurements, values below LOD were 13.8% for DMA, 49.9% for arsenobetaine and over 80% for arsenite, arsenate and MMA. Arsenite, arsenate, MMA and DMA were summed under inorganic As. Total urinary speciated As was calculated by summing arsenite, arsenate, MMA, DMA and arsenobetaine.

Blood pressure and diagnosis of HDP

The maternal systolic (SBP) and diastolic (DBP) blood pressures were measured during each clinic visit by the staff using a sphygmomanometer. Two measures of blood pressure were taken about one minute apart and averaged for each visit. Blood pressure was assessed in a sitting position, with the cuffed arm resting on a desk at the level of the heart. The Korotkoff phase V (disappearance) was used for DBP measurement. The Korotkoff phase IV (muffling) was used only when a phase V was absent.

The mean arterial pressure of the two measures taken at each visit was determined for four time periods: visit 1 (6 to 13 weeks), visit 2 (16 to 21 weeks), visit 3 (32 to 34 weeks) and visit 4 (after admission for delivery). The results were used according to this formula: (SBP + 2 DBP)/3, obtaining one value for mean arterial pressure. Blood pressure data following admission for delivery were abstracted from the hospital chart. The diagnostic criteria for HDP were based on the Society of Obstetricians and Gynaecologists of Canada Guidelines (Magee et al. 2014). According to this guideline, a diagnosis of hypertension is based on the average of two measurements of SBP ≥ 140 mmHg and/or DBP ≥ 90 mmHg, taken at least 1 minute apart in MIREC study and using the same arm. *De novo* appearance of hypertension at ≥ 20 weeks of gestation with or without PE defined GH overall. The presence of proteinuria (proteinuria 24h ≥

300 mg/24h) or maternal complications defines PE whereas their absence defines GH without preeclampsia (GH in this study) (Magee et al. 2014). In this study, four categories were established for HDP status: Normotensive, GH overall and its subcategories GH without PE and PE. Gestational age (GA) in weeks was based on last menstrual period and/or early ultrasound result. An algorithm summarizing the steps for the diagnosis of HDP is available in supplemental material (see Fig. S1).

Covariates

Data on potential covariates were derived from questionnaires administered at the first, second and third visits as well as from medical chart reviews. The following variables were analyzed as covariates: maternal age at delivery in years (continuous form), parity (multiparous, nulliparous), ethnicity (caucasian, non-caucasian), body mass index before pregnancy (BMI) (weight in kg divided by height squared in meters and categorized as underweight, normal weight, overweight, obese), weight gain during pregnancy (kg) (difference between last weight measured prior to delivery and weight measured at first trimester visit), education (university, college, less than college), household income ($<15,000, 16,000-35,000, 36,000-45,000, 46,000-55,000, 56,000-65,000, 66,000-75,000, 76,000-90,000, > 90,000), maternal smoking (never, quit before pregnancy, quit during pregnancy, current smoker), any alcohol consumption during the 3 months before visit 1 (no, yes), and fish (excluding shellfish) consumption (relative total quantity serving size per day calculated from the food frequency questionnaire by Morisset et al. (2016)). To account for the effect of urinary dilution, specific gravity was also considered as a covariate in models of urinary speciated As.

Statistical analysis

Descriptive statistics were performed to analyze participant characteristics according to HDP status using Student's t-test, the Mann-Whitney, Kruskal-Wallis or chi-square tests. Geometric means (GM) (with 95% confidence intervals (CI)) and categorical distributions of metals were determined for each outcome category (normotensive, GH overall, GH and PE). Levels of metals were compared using Mann-Whitney and Kruskal-Wallis and tertiles were compared using the chi-square test. Spearman correlations were performed to test the correlations between first and third trimester blood metals and among the metals at each trimester.

We used several analytic approaches to study the relationships between metal exposure and risk of HDP. Adjusted Odd ratios (aOR) and their corresponding 95% Confidence intervals (CI) were estimated using logistic regression models to test the relationship between metals and GH overall versus normotensive status or using multinomial regression models with HDP defined according three mutually exclusive groups (normotensive, GH and PE). All metals were individually tested in each model as a continuous as well as a categorical variable (tertiles) to consider different alternatives for modeling exposure. The results were grouped in the tables according to the type of specimen collected (blood versus urine) and the trimester of specimen collection. The p-value (p) <0.05 was considered to indicate statistical significance. In order to control for potential confounding bias, covariates were selected either *a priori* on the basis of evidence from the literature (maternal age) or according to the change-in-estimate method. All covariates among the list presented above were examined individually and those which changed the OR by +/-10% for the relationship between metals and HDP were considered as confounders and were included in each corresponding multivariate model. In order to increase power in the multivariate models, covariates were considered in a continuous form when possible and missing

data were ignored. This may have reduced the number of women in certain multivariate models but as missing data were rare and considered as "missing completely at random (MCAR)", this did not have significant impact on the results.

We also investigated the associations between metals and HDP through the potential modification effect of infant sex. We were not able to test the interaction according to maternal smoking status because of the small number of smokers in our study. The results were stratified if the interaction term was significant at $p < 0.05$. In addition we conducted two sensitivity analyses: 1) with fish consumption in the model of mercury and 2) including all blood metals in the same model at each trimester.

In a separate set of analyses, we also explored the relationship between concurrent measures of metals and mean blood pressure using linear generalized estimating equation models, which take into account the clustering within each individual caused by the repeated-measurements design. Models incorporated a first-order autoregressive correlation pattern for the repeated events. The mean arterial pressure was estimated for each woman at each visit (from each individual measure collected in the corresponding visit) and was used as continuously measured outcomes in the linear generalized estimating equations regression models in order to estimate the adjusted beta coefficients (ß) and their corresponding 95%CI. Unlike to HDP, mean blood pressure took into account the measurement of blood pressure in the first trimester. Metals were transformed into their natural-logarithm (ln) to approximate a normal distribution. Linear generalized estimating equation models were developed using concurrent measures of metals and mean arterial pressure at visits 1 and 3. This model allowed the examination of the direct impact of each metal taken at the same time window that blood pressure was measured. It also allowed

examining the association with the blood pressure and not only with the pathologic outcome of hypertension in pregnant women. Since urinary As was measured only once during the first visit, we used linear regression to analyze its association with blood pressure measured at visit 1. All statistical analyses were performed with IBM SPSS Statistics Version 22, SAS 9.4 for Windows, StataSE 14 and R for Windows 3.2.2.

Results

The majority (82%) of the participants were Caucasian, university educated, multiparous, non-smokers, had a household income more than $90,000, and did not consume alcohol in the 3 months before visit 1. The number of women with GH overall was 187 (9.8%) and within this group, 129 (6.8%) had GH without PE, while 58 (3%) had PE. Women with chronic hypertension (n = 58 (3%)) or without data on hypertension status (n = 34 (1.8%)) were treated as missing data with respect to HDP. The study population characteristics are shown in Table 1. The mean maternal age was similar across study groups. The proportion of obesity was significantly higher among women with PE (44.2%) compared with normotensive women (11.6%). Weight gain during pregnancy was also higher in women who developed HDP than among normotensive women. Fish consumption and birth weight were higher in normotensive women. Ethnicity, BMI, education, household income, parity, fish consumption, and birth weight were also significantly associated with HDP.

Spearman correlations between the first and third trimester concentrations of maternal blood metals were significant: Spearman's rho (r) = 0.41 ($p < 0.001$), r = 0.75 ($p < 0.001$), r = 0.64 ($p < 0.001$), r = 0.76 ($p < 0.001$) and r = 0.66 ($p < 0.001$), respectively for As, Pb, Cd, Hg and Mn (Table S1). Similarly first trimester concentrations of blood and urinary As were significantly and

positively correlated for arsenobetaine (r = 0.55, p <0.001), for total urinary As (r = 0.43, p <0.001), for DMA (r = 0.23, p <0.001) and for inorganic As (r = 0.23, p <0.001) (Table S2). Correlations between metals measured during the same trimester were generally low with the highest correlation being between blood As and Hg (r = 0.39) in the third trimester (Tables S3-S5).

In the 1st and 3rd trimesters GM blood Hg concentrations were significantly lower in women who developed GH overall (0.36 µg/l), GH (0.37 µg/l) or PE (0.34 µg/l) compared to women who remained normotensive (0.50 µg/l) (Table 2, Fig. S2). Both GM blood Pb and Mn concentrations at 1st trimester were significantly lower in women who went on to develop HDP than those who remained normotensive women. Maternal concentrations of blood Cd and As were not significantly associated with HDP. The percentage distribution of tertiles of metal concentrations according to HDP is presented in Table S6.

In the multivariate analysis, a significantly lower risk of GH overall (aOR = 0.62; 95% CI 0.39, 0.98) in the 3rd trimester was observed at the 3rd tertile (> 0.82 µg/l) of blood Hg (Table 3). When 3rd trimester blood Hg was examined as a continuous variable, a lower risk of GH overall was observed (aOR = 0.69; 95% CI 0.50, 0.96) (Table S8), even when controlling for other metals in the model (aOR = 0.66; 95% CI 0.47, 0.93) (Table S10). Blood Hg was not significantly associated with blood pressure in the cross-sectional analysis (Table 5).

First trimester blood manganese (> 9.89 µg/l versus <7.69 µg/l) was associated with a lower risk of developing GH overall (aOR = 0.68; 95% CI 0.46, 0.99) and PE (aOR = 0.48; 95% CI 0.23, 0.98) (Table 3) and was still associated with a significantly lower risk when examined as a continuous variable, adjusting for the other metals (Table S10). When stratified by infant sex,

the risk of GH overall for those in the 3rd tertile for manganese was significantly lower in women delivering male infants (aOR = 0.41; 95% CI 0.24, 0.70) than female infants (aOR = 1.27; 95% CI 0.69, 2.34) (Table 4). In contrast, when blood Mn was modeled with concurrent blood pressure, a significant positive association was observed (aß=1.52; 95% CI: 0.80-2.24) (Table 5).

First trimester blood As (> 0.97 versus <0.60 µg/l) was associated with an almost tripling of the risk of PE (OR = 2.75; 95% CI 1.13, 6.73), adjusted for the other 4 metals (Table S10). In the 3rd trimester, a 1-unit increase in ln-blood As was associated with a significant increase in the risk of PE (aOR = 1.16; 95% CI 1.03, 1.30) (Table S8), even after adjusting for other metals (OR = 1.13; 95% CI 1.00, 1.28) (Table S10). The risk of GH overall associated with As was significantly higher among women carrying male fetuses (aOR = 1.85 (95%CI: 1.02, 3.36) for As level 0.60-0.97 µg/l versus < 0.60 µg/l) than those carrying female fetuses (aOR=0.78 (0.44, 1.37)) (Table 4). Concurrent measures of blood As and blood pressure displayed a significant negative association (beta = - 0.59; 95% CI -0.92, -0.26) (Table 5).

Neither urinary DMA, arsenobetaine nor inorganic As were significantly associated with the risk of HDP; however, a significant negative association was observed between concurrent measures of blood pressure and DMA (Table 6). The second tertile of total urinary As was associated with a lower risk of PE (Table 3) (this result may be due to a type I error). No significant associations were observed between blood Pb or Cd concentrations and the risk of HDP, although the risks for 3rd trimester concentrations were above 1.0 for Pb (Table 3). Also the association between Pb, Cd or Hg concentrations and HDP was not modified by infant sex.

Discussion

Blood metals and HDP

Mn and HDP

In our study, the GM of Mn concentrations (measured at first trimester) were lower in patients who developed GH overall (8.43 µg/l) than in normotensive women (8.83 µg/l). The highest tertile of Mn (> 9.89 µg/l) was associated with a decrease in risk of GH overall and PE risk. This finding is consistent with a previous case-control study (N = 108) that reported that serum mean Mn concentrations were significantly lower in PE (0.08 µg/l) than in control women (0.14 µg/l) (Sarwar et al. 2013). Similar results were also observed by Al-Jameil et al. (2014) and Maduray et al. (2017). However, our findings contrast with those reporting an association between Mn and the higher risk of HDP. In a cohort study of 364 healthy pregnant women, Vigeh et al. (2013) observed that maternal blood Mn concentrations measured in the first (mean = 18.6 µg/l) and second trimesters (mean = 18.9 µg/l), were associated with a higher risk of GH. Third trimester results were not statistically significant. Similar results were reported by Vigeh et al. (2006). This contrast in results may be explained by the lower concentrations of Mn in our study (GM around 8 µg/l) compared to that of Vigeh et al. (2013). It is possible that at higher levels, Mn is toxic and could cause GH (an inverted U dose-response shape). In the general population, Lee and Kim (2011) found an association between Mn and higher SBP and DBP (GM was 1.33 mg/dl in hypertensive participants). While our data suggest the importance of adequate levels of manganese for reducing the risks of GH and PE, data from other studies suggest that if the levels are too high, this may increase the risk of HDP.

We also observed in our study that fetal sex had a significant impact on the association between blood Mn and HDP. Although no study examining the potential modifying effect of fetal

sex on the association between HDP and Mn was found, our result was consistent with that of Lee et al. (2015) who found a lower risk of SBP in men than in women exposed to Mn.

When concurrent measures of Mn levels and blood pressure were analyzed, we found that blood Mn was associated with an increased blood pressure. Different results obtained with these models may indicate that metal exposures might have different relationships to blood pressure compared to HDP which are two different outcomes. It is also possible that measures of Mn may have coincided with the time of its maximal effect on blood pressure. To our knowledge, ours is the first study to examine concurrent measurements of Mn and blood pressure among pregnant women.

Mn is an essential element (Avila et al. 2013) and may have an antioxidant function (Candas and Li 2014; Fukai and Ushio-Fukai 2011). It has been suggested that antioxidant may be effective in the prevention of preeclampsia (Raijmakers et al. 2004). On the other hand, low nitric oxide (NO) may be associated with PE (Choi et al. 2002; Lowe 2000) and GH (Wang et al. 2000). Mn can induce increased levels of arginine (Santos et al. 2012) which is converted into NO by nitric oxide synthetase (Avila et al. 2013; Boucher et al. 1999). NO plays an important role in the control of blood pressure (Boucher et al. 1999; Hermann et al. 2006), GH (Wang et al. 2000) and PE (Choi et al. 2002). This mechanism suggests that Mn can be associated with a decreased risk of GH and PE by increasing NO levels. In contrast, some studies have showed that Mn can increase oxidative stress (Chtourou et al. 2011; Cordova et al. 2013; Liu et al. 2013) which can lead to alteration of nitric oxide (NO) bioavailability and endothelial dysfunction (which can contribute to hypertension) (Schulz et al. 2011).

As and HDP

Our findings suggest that blood As (measured at third trimester or first trimester when controlled for the other metals) was associated with a higher risk of PE. To our knowledge, there are no previous cohort studies in pregnant women assessing the association between blood As and HDP. However, our results are consistent with those of a case-control study (n = 398 women) where the authors found an association between hair As and higher risk of hypertension (OR = 2.55; 95% CI 1.55, 4.20) (Yu et al. 2017) but not those from another case-control study (Maduray et al. 2017) which did not find an association between hair or serum As and PE. A systematic review of studies conducted in the general population (Abhyankar et al. 2012) found an increased risk of high blood pressure for those exposed to high As concentrations from drinking water (OR = 2.57; 95% CI 1.56, 4.24) compared to those with a lower As concentrations.

Neither urinary DMA, arsenobetaine or inorganic As were significantly associated with the risk of HDP. Our result was consistent with those of others studies, both in pregnant women (Sandoval-Carrillo et al. 2016) and in the general population (Jones et al. 2011; Li et al. (2013). In contrast, Shiue and Hristova (2014) found an association between blood pressure and DMA concentrations (mean = 5.72 μg/l). Similar results were found by Shiue (2014a, 2014b). This disparity in results may be due to the lower concentrations of DMA in our study (2.30 μg/l was the highest GM).

Examining concurrent measures of As and blood pressure, we found that blood As as well as urinary DMA were associated with a decreased blood pressure. As explained in Mn section, the difference with HDP results may be explained by the characteristics of both issues. Also concurrent measures of As may have coincided with the time of its minimal effect on blood

pressure. Using linear mixed effects modeling in their study (n = 514 pregnant women), Farzan et al. (2015) observed an association between As measured in urine and increased blood pressure. The levels of As was lower in our study (1.50 to 89.51 µg/l) compared to Farzan et al. (2015) (0.35 to 288.5 µg/l).

Arsenic may induce endothelial dysfunction through suppression of vascular endothelium integrity, inactivation of the endothelial NO synthase which reduces NO bioavailability and increases oxidative stress (Jomova et al. 2011). Increased oxidative stress and NO deficiency are associated with PE (Davidge 1998). Also, As may increase interleukin-6 (Ma et al. 2012; Wang et al. 2012) which can promote endothelial dysfunction and the pathogenesis of PE (Lockwood et al. 2008).

Hg and HDP

In our study, blood Hg (measured at third trimester) was associated with a lower risk of GH overall. The sensitivity analysis with fish consumption in the model did not change the magnitude of this association. Hg tertiles measured at first trimester were not associated with the risk of HDP. Blood GM Hg concentrations were lower in women with GH overall (0.36 µg/l), GH (0.37 µg/l) and PE (0.34 µg/l) compared to normotensive women (0.50 µg/l). In accordance with our results, a cross-sectional study of 263 pregnant women, found that inorganic Hg (GM = 0.13 µg/l) was associated with a lower SBP (-1.18 mmHg (-3.72, 1.35)) and lower pulse pressure (-2.51 mmHg (-4.49, -0.53)) in contrast to methylmercury (GM = 0.95 µg/l) which was associated with higher SBP (Wells et al. 2017). In the general population, an analysis of the U.S. National Health and Nutrition Examination Survey (NHANES) 2003-2006, found that urinary Hg was associated with a decreased risk of hypertension (OR = 0.87; 95% CI 0.78, 0.99) and no

association was shown for blood Hg (Park et al. 2013). Others authors reported mixed findings (Goodrich et al. 2013; Mozaffarian et al. 2012; Nielsen et al. 2012).

Hg measured in the first trimester has been associated with an increased risk of high MMP-9 whereas third trimester Hg was associated with high MMP-7 (Au et al. 2016). Hg can activate MMP-2 and MMP-9 (Jacob-Ferreira et al. 2009). MMP-7 might activate MMP-9 (Galewska et al. 2010). Higher levels of MMP-2 and MMP-9 are implicated in vasodilation, placentation, and uterine expansion during normal pregnancy whereas their lower levels are associated with decreased vasodilation, increased vasoconstriction, hypertension in pregnancy and PE (Chen and Khalil 2017). This mechanism suggests that Hg can be associated with a decreased risk of hypertension in pregnant women by increasing MMP-2 and MMP-9 levels.

Pb and HDP

In our study, Pb measured in blood at first or third trimester was not associated with HDP, although the risks were above 1.0 in the third trimester. Blood Pb GM measured at first but not third trimester was lower in GH overall (0.57 µg/dl), GH (0.58 µg/dl) and PE (0.57 µg/dl) compared to normotensive women (0.63 µg/dl). In accordance to ours results, Maduray et al. (2017) found similar concentrations of serum Pb in preeclamptic (0.20 ± 0.17 µg/l) and control women (0.16 ± 0.21 µg/l). The substantially lower blood Pb levels in our study compared with others may provide an explanation for the disparity in results. For example, the Pb levels for PE groups were higher in Ikechukwu et al. (2012) (mean = 60.2 µg/dl), Motawei et al. (2013) (mean = 37.68 µg/dl) and in Sowers et al. (2002) (mean = 1.2 µg/dl) studies or for GH groups in Magri et al. (2003) (mean = 9.6 µg/dl), Rabinowitz et al. (1987) (mean = 6.9 µg/dl), Vigeh et al. (2004) (mean = 5.7 µg/dl) and in Yazbeck et al. (2009) (GM = 1.9 µg/dl) studies. Some methodological

differences may also explain the discrepancy between our study and that of Wells et al. (2011) who found an association between cord blood Pb (GM = 0.66 µg/dl) and elevations in blood pressure. Blood pressure measurements in the Wells et al. (2011) study were taken during labor and delivery, a period known to be stressful (Costa et al. 1988; Irestedt et al. 1982). In addition, only one measurement was taken which could introduce measurement error and potentially affect the validity of their results.

We did not find a significant association between concurrent measures of blood Pb and blood pressure. In accordance with our findings, González-Muñoz et al. (2010) showed no association between Pb and blood pressure. Similarly, Mordukhovich et al. (2012) found no association between Pb and SBP in men while Bushnik et al. (2014) reported an association between blood Pb (GM = 1.74 µg/dl) and blood pressure in men but not in women. This discrepancy with our findings may be explained by the lower concentrations of blood Pb in our study.

Cd and HDP

No association was found between Cd measured in blood at first or third trimester and HDP risk nor when concurrently measured with blood pressure. Blood Cd levels in our study (GM 0.2 µg/l) were lower than those reported by others which found an association with GH (mean = 1.9 µg/l (Kosanovic and Jokanovic 2007)) or hypertension (mean = 1.67 µg/l (Eum et al. 2008)). In concordance with our results, one study reported no association between Cd and GH (Yazbeck et al. 2009) and others reported no association between Cd and hypertension (Al-Saleh et al. 2006; Shiue and Hristova 2014). In contrast, Laine et al. (2015) found a relationship between Cd in placenta (mean = 3.7 ng/g) and PE.

Strengths and limitations of the study

The main strengths of this study are the large sample size (n = 1909) and the prospective cohort design which increases the validity of study findings. All laboratory measurements were performed by a national reference laboratory. Clinical outcome measures were standardized by trained field staff. In addition, the multi-centre nature of the study provides a pan-Canadian exposure profile and has potential for generalizability. However, the average higher socio-economic status of participants may reduce the generalizability to socio-economically different populations. In addition, maternal metal concentrations were not linked to external exposure levels in various media, so could not be extrapolated to external doses that would be needed for the purpose of a quantitative human health risk assessment.

Conclusion

Higher concentrations of blood Mn and Hg were associated with lower risk of HDP, while blood As was associated with an elevated risk. These associations did not change when all metals were included in the same model. When we analyzed the associations between blood metal concentrations and concurrent measures of blood pressure, we found that blood As concentration (and DMA in urine) was inversely associated with blood pressure, while blood Mn was positively associated with higher blood pressure. Finally, the association between blood Mn (measured at first trimester) and HDP was modified by infant sex. Blood Mn was associated with lower risk of GH overall and GH in women carrying a male fetus. Further studies are required to determine if these results can be replicated in other similar populations with current low levels of exposure.

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Table 11. Participant characteristics according to presence or absence of hypertensive disorders of pregnancy (HDP)

Characteristics	Normotensive n = 1630	GH overall n = 187	P[a]	GH n = 129	Preeclampsia n = 58	P[b]
Maternal Age (years), mean ± SD	32.9 ± 5.0	32.5 ± 5.2	0.378	32.8 ± 4.9	31.9 ± 5.9	0.371
Weight gain, mean ± SD	14.86 ± 6.01	16.23 ± 8.04	0.008	15.32 ± 7.09	18.28 ± 9.61	0.001
Pre-pregnancy BMI, n (%)[c]			<0.001			<0.001
Underweight	46 (3.0%)	3 (1.8%)		2 (1.7%)	1 (1.9%)	
Normal weight	976 (64.6%)	64 (38.3%)		48 (41.7%)	16 (30.8%)	
Overweight	315 (20.8%)	45 (26.9%)		33 (28.7%)	12 (23.1%)	
Obesity	175 (11.6%)	55 (32.9%)		32 (27.8%)	23 (44.2%)	
Ethnicity			0.008			0.021
Caucasian	1392 (85.4%)	173 (92.5%)		121 (93.8%)	52 (89.7%)	
Non-caucasian	238 (14.6%)	14 (7.5%)		8 (6.2%)	6 (10.3%)	
Education			0.010			0.002
University	1037 (63.7%)	98 (52.4%)		74 (57.4%)	24 (41.4%)	
College	424 (26.0%)	63 (33.7%)		42 (32.6%)	21 (36.2%)	
Less college	167 (10.3%)	26 (13.9%)		13 (10.1%)	13 (22.4%)	
Income in $CAN			0.004			0.006
≤15,000	58 (3.7%)	12 (6.6%)		7 (5.5%)	5 (9.1%)	
16,000-35,000	136 (8.8%)	12 (6.6%)		5 (3.9%)	7 (12.7%)	
36,000-45,000	80 (5.2%)	11 (6.0%)		7 (5.5%)	4 (7.3%)	
46,000-55,000	82 (5.3%)	10 (5.5%)		6 (4.7%)	4 (7.3%)	
56,000-65,000	103 (6.6%)	19 (10.4%)		15 (11.8%)	4 (7.3%)	
66,000-75,000	132 (8.5%)	20 (11.0%)		17 (13.4%)	3 (5.5%)	
76,000-90,000	303 (19.5%)	44 (24.2%)		32 (25.2%)	12 (21.8%)	
>90,000	657 (42.4%)	54 (29.7%)		38 (29.9%)	16 (29.1%)	
Parity			0.008			0.001
Multiparous	941 (57.8%)	89 (47.6%)		70 (54.3%)	19 (32.8%)	
Nulliparous	687 (42.2%)	98 (52.4%)		59 (45.7%)	39 (67.2%)	

Maternal smoking			0.487			0.392
Never	1007 (61.8%)	112 (59.9%)		82 (63.6%)	30 (51.7%)	
Quit before Pregnancy	518 (31.8%)	67 (35.8%)		42 (32.6%)	25 (43.1%)	
Quit during Pregnancy	17 (1.0%)	2 (1.1%)		2 (1.6%)	0 (0.0%)	
Current Smoking	87 (5.3%)	6 (3.2%)		3 (2.3%)	3 (5.2%)	
Alcohol consumption 3months before visit1			0.376			0.344
No	943 (57.9%)	102 (54.5%)		74 (57.4%)	28 (48.3%)	
Yes	685 (42.1%)	85 (45.5%)		55 (42.6%)	30 (51.7%)	
Fish consumption (serving/day), median (IQR)	0.10 (0.03, 0.17)	0.07 (0.00, 0.14)	0.005	0.07 (0.03, 0.14)	0.07 (0.00, 0.14)	0.010
Infant sex			0.670			0.696
Female	775 (47.6%)	86 (46.0%)		57 (44.2%)	29 (50.0%)	
Male	852 (52.4%)	101 (54.0%)		72 (55.8%)	29 (50.0%)	
Birth weight (gram), mean ± SD	3451.1 ± 522.0	3296.8 ± 705.6	<0.001	3338.5 ± 614.1	3204.1 ± 874.8	<0.001

Numbers did not equal 1909 women because of missing data on covariates or HDP.
HDP=hypertensive disorders of pregnancy. GH overall=gestational hypertension with or without preeclampsia. GH= gestational hypertension without preeclampsia. BMI= Body mass index. SD = standard deviation. IQR= interquartile range
[a] p-values for the association with Normotensive and GH overall: T-test or Mann-Whitney for continuous variables, Chi-square test for categorical variables
[b] p-values for the association with Normotensive, GH and Preeclampsia : ANOVA or Kruskal-Wallis for continuous variables, Chi-square test for categorical variables
[c] Values are n (%), unless otherwise started

Table 12. Geometric mean (GM) of blood metal concentrations according to HDP

Metals	Normotensive		GH overall			GH		Preeclampsia		
	n[c]	GM (95% CI)	n	GM (95% CI)	P[a]	n	GM (95% CI)	n	GM (95% CI)	P[b]
Blood first trimester										
Arsenic (µg/l)	1598	0.73 (0.70, 0.76)	181	0.71 (0.64, 0.80)	0.715	126	0.67 (0.58, 0.76)	55	0.83 (0.68, 1.03)	0.202
Lead (µg/dl)	1598	0.63 (0.61, 0.64)	181	0.57 (0.54, 0.61)	0.005	126	0.58 (0.53, 0.63)	55	0.57 (0.50, 0.64)	0.018
Cadmium (µg/l)	1598	0.22 (0.21, 0.23)	181	0.20 (0.17, 0.22)	0.245	126	0.19 (0.17, 0.22)	55	0.21 (0.16, 0.28)	0.390
Mercury (µg/l)	1598	0.62 (0.58, 0.65)	181	0.50 (0.42, 0.58)	0.013	126	0.51 (0.42, 0.61)	55	0.47 (0.34, 0.65)	0.044
Manganese (µg/l)	1598	8.83 (8.70, 8.97)	181	8.43 (8.04, 8.85)	0.030	126	8.54 (8.07, 9.05)	55	8.18 (7.49, 8.94)	0.065
Urinary first trimester (µg As/l)										
DMA	1588	2.23 (2.12, 2.35)	185	2.26 (1.94, 2.62)	0.909	128	2.30 (1.94, 2.72)	57	2.16 (1.57, 2.97)	0.932
Arsenobetaine	1589	1.28 (1.18, 1.38)	185	1.33 (1.05, 1.69)	0.828	128	1.19 (0.93, 1.54)	57	1.70 (1.01, 2.88)	0.802
Inorganic arsenic	1588	3.89 (3.75, 4.03)	185	3.97 (3.56, 4.41)	0.812	128	3.99 (3.53, 4.51)	57	3.92 (3.15, 4.87)	0.844
Total arsenic	1588	6.18 (5.88, 6.49)	185	6.36 (5.44, 7.43)	0.944	128	5.99 (5.09, 7.05)	57	7.28 (5.10, 10.38)	0.994
Blood third trimester										
Arsenic (µg/l)	1444	0.65 (0.62, 0.68)	164	0.58 (0.51, 0.67)	0.097	114	0.56 (0.48, 0.66)	50	0.63 (0.47, 0.84)	0.197
Lead (µg/dl)	1444	0.57 (0.55, 0.58)	164	0.58 (0.53, 0.62)	0.746	114	0.56 (0.50, 0.62)	50	0.62 (0.55, 0.71)	0.439
Cadmium (µg/l)	1444	0.20 (0.19, 0.21)	164	0.19 (0.17, 0.21)	0.372	114	0.18 (0.16, 0.20)	50	0.21 (0.17, 0.26)	0.410
Mercury (µg/l)	1444	0.50 (0.47, 0.53)	164	0.36 (0.31, 0.42)	0.001	114	0.37 (0.31, 0.45)	50	0.34 (0.24, 0.46)	0.002
Manganese (µg/l)	1444	12.27 (12.06, 12.49)	164	11.86 (11.20, 12.55)	0.300	114	11.89 (11.07, 12.78)	50	11.77 (10.73, 12.91)	0.557

Numbers did not equal 1909 women because of missing data on metals or HDP.
HDP=hypertensive disorders of pregnancy. GH overall=gestational hypertension with or without preeclampsia. GH= gestational hypertension without preeclampsia. DMA= dimethylarsinic acid. GM= Geometric Mean. CI= confidence interval.
[a]Mann-Whitney test for the association between metals and Normotensive and GH overall.
[b] Kruskal-Wallis test for the association between metals and Normotensive, GH and preeclampsia.
[c] excluded women with missing data

Table 13. Associations between metal concentrations (categorical form) and HDP according to the type of specimen tested (blood versus urine) and trimester of measurement

Metals	GH overall			GH	Preeclampsia
	n	**Adjusted OR (95% CI)[a]**	**n**	**Adjusted OR (95% CI)[b]**	**Adjusted OR (95% CI)[b]**
Blood first trimester					
Arsenic (µg/l)[c]	1621		1778		
<0.60		Reference		Reference	Reference
0.60 - 0.97		1.17 (0.79, 1.74)		1.03 (0.67, 1.59)	1.27 (0.65, 2.50)
> 0.97		1.02 (0.68, 1.54)		0.85 (0.54, 1.34)	1.35 (0.69, 2.65)
Lead (µg/dl)[d]	1645		1570		
<0.50		Reference		Reference	Reference
0.50 - 0.75		1.08 (0.74, 1.58)		1.44 (0.92, 2.26)	0.53 (0.26, 1.10)
> 0.75		0.70 (0.45, 1.11)		0.77 (0.44, 1.35)	0.66 (0.31, 1.39)
Cadmium (µg/l)[e]	1459		1459		
<0.16		Reference		Reference	Reference
0.16 - 0.27		1.15 (0.75, 1.76)		1.07 (0.66, 1.75)	1.36 (0.64, 2.91)
> 0.27		0.82 (0.50, 1.33)		0.79 (0.45, 1.39)	0.87 (0.37, 2.04)
Mercury (µg/l)[f]	1593		1591		
<0.42		Reference		Reference	Reference
0.42 - 1.06		1.30 (0.86, 1.95)		1.21 (0.76, 1.93)	1.59 (0.77, 3.26)
> 1.06		0.89 (0.56, 1.42)		0.83 (0.48, 1.43)	1.09 (0.47, 2.54)
Manganese (µg/l)[g]	1778		1778		
<7.69		Reference		Reference	Reference
7.69 - 9.89		0.98 (0.67, 1.42)		0.95 (0.61, 1.49)	1.03 (0.55, 1.92)
> 9.89		0.68 (0.46, 0.99)*		0.77 (0.49, 1.21)	0.48 (0.23, 0.98)*
Urinary first trimester (µg As/l)					
DMA[h]	1772		1772		
<1.50		Reference		Reference	Reference
1.50 - 3.67		0.77 (0.49, 1.20)		0.85 (0.51, 1.43)	0.59 (0.26, 1.31)

> 3.67		0.73 (0.43, 1.24)		0.66 (0.35, 1.25)	0.89 (0.36, 2.19)
Arsenobetaine[i]	1772		1772		
<0.38		Reference		Reference	Reference
0.38 - 1.95		1.14 (0.74, 1.74)		1.13 (0.69, 1.87)	1.15 (0.55, 2.41)
> 1.95		0.93 (0.65, 1.33)		0.91 (0.60, 1.40)	0.98 (0.52, 1.82)
Inorganic arsenic[j]	1772		1772		
<2.67		Reference		Reference	Reference
2.67 - 4.87		0.81 (0.52, 1.27)		0.93 (0.55, 1.57)	0.57 (0.26, 1.28)
> 4.87		0.78 (0.46, 1.32)		0.76 (0.40, 1.43)	0.84 (0.34, 2.06)
Total arsenic[k]	1772		1772		
<3.45		Reference		Reference	Reference
3.45 - 7.79		0.77 (0.50, 1.18)		1.05 (0.63, 1.73)	0.36 (0.16, 0.80)**
> 7.79		0.72 (0.46, 1.13)		0.81 (0.47, 1.40)	0.57 (0.27, 1.22)
Blood third trimester					
Arsenic (µg/l)[l]	1607		1447		
<0.51		Reference		Reference	Reference
0.51 - 0.90		1.04 (0.71, 1.52)		0.99 (0.61, 1.60)	2.04 (0.94, 4.46)
> 0.90		0.73 (0.48, 1.10)		0.71 (0.40, 1.26)	1.63 (0.68, 3.91)
Lead (µg/dl)[m]	1490		1474		
<0.46		Reference		Reference	Reference
0.46 - 0.68		1.17 (0.77, 1.80)		1.34 (0.82, 2.20)	1.05 (0.47, 2.36)
> 0.68		1.27 (0.82, 1.98)		1.27 (0.73, 2.21)	1.88 (0.86, 4.11)
Cadmium (µg/l)[n]	1410		1386		
<0.16		Reference		Reference	Reference
0.16 - 0.25		0.83 (0.54, 1.26)		0.94 (0.56, 1.58)	1.31 (0.60, 2.86)
> 0.25		0.81 (0.51, 1.27)		0.94 (0.55, 1.62)	1.19 (0.52, 2.72)
Mercury (µg/l)[o]	1490		1374		
<0.36		Reference		Reference	Reference
0.36 - 0.82		0.75 (0.50, 1.14)		0.75 (0.45, 1.26)	1.03 (0.48, 2.21)
> 0.82		0.62 (0.39, 0.98)*		0.67 (0.37, 1.21)	0.74 (0.29, 1.91)
Manganese (µg/l)[p]	1607		1607		

<10.99		Reference		Reference	Reference
10.99 - 14.29		0.70 (0.47, 1.04)		0.74 (0.46, 1.18)	0.62 (0.31, 1.26)
> 14.29		0.80 (0.54, 1.17)		0.81 (0.51, 1.27)	0.78 (0.40, 1.50)

Numbers did not equal 1909 women because of missing data on metals, covariates or HDP on multivariate models.
HDP=hypertensive disorders of pregnancy. GH= gestational hypertension without preeclampsia. GH overall=gestational hypertension with or without preeclampsia. CI= confidence interval. OR= odds ratio. DMA= dimethylarsinic acid.

[a] **Logistic regression model (between each metal and GH overall versus Normotensive) adjusted for:**

[c] weight gain, maternal age

[d] BMI, mercury, maternal age

[e] education, fish consumption, maternal smoking, household income, BMI, weight gain, maternal age

[f] BMI, weight gain, maternal age

[g] maternal age

[h,i,j,k] specific gravity, maternal age

[l] maternal age

[m] BMI, mercury, maternal age

[n] education, maternal smoking, household income, weight gain, maternal age

[o] BMI, maternal age

[p] maternal age

[b] **multinomial logistic regression model (between each metal and GH and preeclampsia versus Normotensive) adjusted for:**

[c] maternal age

[d] BMI, household income, mercury, maternal age

[e] education, BMI, maternal smoking, household income, fish consumption, weight gain, maternal age

[f] education, BMI, weight gain, maternal age

[g] maternal age

[h,i,j,k] specific gravity, maternal age

[l] BMI, mercury, weight gain, maternal age

[m] ethnicity, education, BMI, maternal smoking, fish consumption, arsenic, mercury, manganese, maternal age

[n] education, BMI, maternal smoking, household income, weight gain, maternal age

[o] education, BMI, household income, fish consumption, weight gain, maternal age

[p] maternal age

[*] $p < 0.05$

[**] $p < 0.03$

Table 14. Adjusted Odds ratios for the associations between metals and HDP, stratified according to infant sex

First trimester blood metals measures (µg/l)	GH overall Adjusted OR (95% CI)[a]		GH Adjusted OR (95% CI)[b]	Preeclampsia Adjusted OR (95% CI)[b]
Female				
Arsenic	0.70 (0.44, 1.11)		0.63 (0.37, 1.10)	0.96 (0.59, 1.57)
Tertile				
<0.60	Reference		Reference	Reference
0.60 - 0.97	0.78 (0.44, 1.37)		0.66 (0.35, 1.26)	1.04 (0.41, 2.61)
> 0.97	0.58 (0.32, 1.08)		0.55 (0.28, 1.10)	0.86 (0.32, 2.31)
Manganese	1.00 (0.92, 1.08)		1.04 (0.95, 1.13)	0.90 (0.77, 1.04)
Tertile				
<7.69	Reference		Reference	Reference
7.69 - 9.89	1.50 (0.82, 2.75)		1.72 (0.79, 3.73)	1.24 (0.50, 3.08)
> 9.89	1.27 (0.69, 2.34)		1.85 (0.87, 3.94)	0.55 (0.19, 1.61)
Male				
Arsenic	1.02 (0.91, 1.15)		0.94 (0.73, 1.21)	1.06 (0.93, 1.20)
Tertile				
<0.60	Reference		Reference	Reference
0.60 - 0.97	1.85 (1.02, 3.36)*		1.54 (0.83, 2.86)	1.67 (0.60, 4.66)
> 0.97	1.76 (0.96, 3.22)		1.27 (0.67, 2.40)	2.11 (0.79, 5.67)
Manganese	0.92 (0.85, 0.99)*		0.91 (0.83, 0.99)*	0.94 (0.82, 1.07)
Tertile				
<7.69	Reference		Reference	Reference
7.69 - 9.89	0.73 (0.45, 1.19)		0.68 (0.38, 1.21)	0.86 (0.37, 2.03)
> 9.89	0.41 (0.24, 0.70)**		0.41 (0.22, 0.75)**	0.42 (0.16, 1.10)

GH overall=gestational hypertension with or without preeclampsia. GH= gestational hypertension without preeclampsia. CI= confidence interval. OR= odds ratio.
[a]**Logistic regression:** between each metal and GH overall versus normotensive
[b]**Multinomial regression**: between each metal and GH and preeclampsia versus normotensive
[a,b] adjusted for same variables as Table 3
* p <0.05 ** p <0.03

Table 15. Concurrent measures of blood metal concentrations and maternal blood pressure in the first and third trimester visits among 1909 pregnant women.

Metals	Number of visit for blood pressure and metals measurements	Blood pressure			
		Unadjusted beta (95% CI)	p	Adjusted beta (95% CI)[a]	p
Ln-arsenic[b]	2	-0.66 (-0.98, -0.34)	<0.001	-0.59 (-0.92, -0.26)	<0.001
Ln-lead[c]	2	-0.62 (-1.25, -0.00)	0.050	-0.15 (-0.80, 0.50)	0.660
Ln-cadmium[d]	2	-0.04 (-0.41, 0.34)	0.853	0.17 (-0.26, 0.60)	0.449
Ln-mercury[e]	2	-0.40 (-0.69, -0.11)	0.007	-0.40 (-0.38, 0.29)	0.796
Ln-manganese[f]	2	1.40 (0.69, 2.12)	<0.001	1.52 (0.80, 2.24)	<0.001

CI= confidence interval. Ln = natural logarithm.

[a] **Linear generalized estimating equations model** (between each blood metal and blood pressure) with first-order autoregressive correlation pattern adjusted for maternal age and:

[b] BMI, mercury

[c] alcohol consumption 3 months before visit1, BMI, weight gain, arsenic, mercury, manganese

[d] education, ethnicity, parity, maternal smoking, household income, fish consumption, BMI, weight gain, arsenic, lead, mercury, manganese

[e] fish consumption, BMI, arsenic, weight gain, lead

[f] lead

Table 16. Cross-sectional analyses of urinary metal concentrations and maternal blood pressure measured during the first trimester visit among 1909 pregnant women.

Metals	Blood pressure			
	Unadjusted beta (95% CI)	**p**	**Adjusted beta (95% CI)[a]**	**p**
Ln-DMA[b]	-0.39 (-0.75, -0.02)	0.037	-1.00 (-1.52, -0.48)	<0.001
Ln-arsenobetaine[c]	-0.14 (-0.38, 0.10)	0.252	0.14 (-0.15, 0.44)	0.344
Ln-inorganic arsenic[d]	-0.37 (-0.90, 0.16)	0.166	-0.32 (-1.05, 0.41)	0.388
Ln-total arsenic[e]	-0.25 (-0.63, 0.12)	0.188	-0.23 (-0.68, 0.21)	0.309

CI= confidence interval. Ln = natural logarithm. DMA= dimethylarsinic acid.
[a] **Linear regression** (between each speciated arsenic and blood pressure) adjusted for maternal age and:
[b] specific gravity
[c] Education, fish consumption, alcohol consumption 3 months before visit1, specific gravity, DMA
[d,e] BMI, fish consumption, specific gravity

Table S1. Correlation between blood metals measured at first and third trimester

Metals measured at first trimester	**Metals measured at third trimester**				
	Arsenic	**Lead**	**Cadmium**	**Mercury**	**Manganese**
Arsenic	r= 0.41**	r= 0.20**	r= 0.05	r= 0.36**	r= 0.06*
Lead	r= 0.13**	r= 0.75**	r= 0.18**	r= 0.23**	r= 0.05*
Cadmium	r= -0.01	r= 0.15**	r= 0.64**	r= 0.01	r= 0.09**
Mercury	r= 0.33**	r= 0.26**	r= 0.08**	r= 0.76**	r= 0.06*
Manganese	r= 0.004	r= 0.01	r= 0.07*	r= 0.01	r= 0.66**

*p <0.05
**p <0.001

Table S2**.** Correlation between arsenic measured in blood and urine at first trimester

Arsenic in urine	**Arsenic in blood**	
DMA	r =0.23	p <0.001
Arsenobetaine	r =0.55	p <0.001
Inorganic arsenic	r =0.23	p <0.001
Total arsenic	r =0.43	p <0.001

Table S3. Correlation between blood metals measured at first trimester

Blood metals	Arsenic	Lead	Cadmium	Mercury	Manganese
Arsenic	r =1	r = 0.17**	r = -0.04	r = 0.38**	r = 0.06*
Lead	r = 0.17**	r =1	r = 0.23**	r = 0.25**	r = 0.10**
Cadmium	r = -0.04	r = 0.23**	r =1	r = 0.05*	r = 0.12**
Mercury	r = 0.38**	r = 0.25**	r = 0.05**	r =1	r = 0.04
Manganese	r = 0.06*	r = 0.10**	r = 0.12**	r = 0.04	r =1

*p <0.05
**p <0.001

Table S4**.** Correlation between blood metals measured at third trimester

Blood metals	Arsenic	Lead	Cadmium	Mercury	Manganese
Arsenic	r =1	r = 0.18**	r = 0.03	r = 0.39**	r = 0.06*
Lead	r = 0.18**	r =1	r = 0.28**	r = 0.29**	r = 0.20**
Cadmium	r = 0.03	r = 0.28*	r =1	r = 0.11**	r = 0.23**
Mercury	r = 0.39**	r = 0.29**	r = 0.11**	r =1	r = 0.10**
Manganese	r = 0.06*	r = 0.20**	r = 0.23**	r = 0.10**	r =1

*p <0.05
**p <0.001

Table S5. Correlation between arsenic speciated in urine measured at first trimester

Arsenic speciated in urine	DMA	Arsenobetaine	Inorganic arsenic	Total arsenic
DMA	r =1	r = 0.50**	r = 0.99**	r =0.87**
Arsenobetaine	r = 0.50**	r =1	r = 0.50**	r = 0.80**
Inorganic arsenic	r = 0.99**	r = 0.50**	r =1	r = 0.87**
Total arsenic	r = 0.87**	r = 0.80**	r = 0.87**	r =1

*p <0.05
**p <0.001

Table S6. Distribution tertiles of metal concentrations according to HDP

Metals	Normotensive n (%)	GH overall n (%)	P [a]	GH n (%)	Preeclampsia n (%)	P [a]
Blood first trimester						
Arsenic (µg/l)						
< 0.60	546 (34.2%)	61 (33.7%)	0.819	45 (35.7%)	16 (29.1%)	0.834
0.60 - 0.97	521 (32.6%)	63 (34.8%)		44 (34.9%)	19 (34.5%)	
> 0.97	531 (33.2%)	57 (31.5%)		37 (29.4%)	20 (36.4%)	
Lead (µg/dl)			0.010			0.007
< 0.50	513 (32.1%)	70 (38.7%)		45 (35.7%)	25 (45.5%)	
0.50 - 0.75	557 (34.9%)	71 (39.2%)		56 (44.4%)	15 (27.3%)	
> 0.75	528 (33.0%)	40 (22.1%)		25 (19.8%)	15 (27.3%)	
Cadmium (µg/l)			0.270			0.508
< 0.16	582 (36.4%)	67 (37.0%)		49 (38.9%)	18 (32.7%)	
0.16 - 0.27	516 (32.3%)	67 (37.0%)		46 (36.5%)	21 (38.2%)	
> 0.27	500 (31.3%)	47 (26.0%)		31 (24.6%)	16 (29.1%)	
Mercury (µg/l)			0.041			0.151
< 0.42	525 (32.9%)	69 (38.1%)		47 (37.3%)	22 (40.0%)	
0.42 - 1.06	527 (33.0%)	67 (37.0%)		46 (36.5%)	21 (38.2%)	
> 1.06	546 (34.2%)	45 (24.9%)		33 (26.2%)	12 (21.8%)	
Manganese (µg/l)			0.078			0.140
< 7.69	441 (27.6%)	58 (32.0%)		39 (31.0%)	19 (34.5%)	
7.69 - 9.89	525 (32.9%)	67 (37.0%)		44 (34.9%)	23 (41.8%)	
> 9.89	632 (39.5%)	56 (30.9%)		43 (34.1%)	13 (23.6%)	
Urinary first trimester (µgAs/l)						
DMA						
< 1.50	540 (34.0%)	62 (33.5%)	0.876	41 (32.0%)	21 (36.8%)	0.631
1.50 - 3.67	536 (33.8%)	60 (32.4%)		46 (35.9%)	14 (24.6%)	
> 3.67	512 (32.2%)	63 (34.1%)		41 (32.0%)	22 (38.6%)	
Arsenobetaine			0.719			0.955

< 0.38	817 (51.4%)	93 (50.3%)		64 (50.0%)	29 (50.9%)	
0.38 - 1.95	247 (15.5%)	33 (17.8%)		23 (18.0%)	10 (17.5%)	
> 1.95	525 (33.0%)	59 (31.9%)		41 (32.0%)	18 (31.6%)	
Inorganic arsenic			0.834			0.615
< 2.67	534 (33.6%)	60 (32.4%)		39 (30.5%)	21 (36.8%)	
2.67 - 4.87	531 (33.4%)	60 (32.4%)		46 (35.9%)	14 (24.6%)	
> 4.87	523 (32.9%)	65 (35.1%)		43 (33.6%)	22 (38.6%)	
Total arsenic			0.964			0.208
< 3.45	535 (33.7%)	64 (34.6%)		39 (30.5%)	25 (43.9%)	
3.45 - 7.79	525 (33.1%)	61 (33.0%)		49 (38.3%0	12 (21.1%)	
> 7.79	528 (33.2%)	60 (32.4%)		40 (31.3%)	20 (35.1%)	
Blood third trimester						
Arsenic (µg/l)						
< 0.51	483 (33.4%)	60 (36.6%)	0.156	47 (41.2%)	13 (26.0%)	0.097
0.51 - 0.90	475 (32.9%)	61 (37.2%)		38 (33.3%)	23 (46.0%)	
> 0.90	486 (33.7%)	43 (26.2%)		29 (25.4%)	14 (28.0%)	
Lead (µg/dl)			0.929			0.515
< 0.46	523 (36.2%)	59 (36.0%)		41 (36.0%)	18 (36.0%)	
0.46 - 0.68	447 (31.0%)	53 (32.3%)		41 (36.0%)	12 (24.0%)	
> 0.68	474 (32.8%)	52 (31.7%)		32 (28.1%)	20 (40.0%)	
Cadmium (µg/l)			0.765			0.936
< 0.16	524 (36.3%)	64 (39.0%)		46 (40.4%)	18 (36.0%)	
0.16 - 0.25	448 (31.0%)	50 (30.5%)		34 (29.8%)	16 (32.0%)	
> 0.25	472 (32.7%)	50 (30.5%)		34 (29.8%)	16 (32.0%)	
Mercury (µg/l)			0.001			0.006
< 0.36	489 (33.9%)	77 (47.0%)		54 (47.4%)	23 (46.0%)	
0.36 - 0.82	465 (32.2%)	50 (30.5%)		32 (28.1%)	18 (36.0%)	
> 0.82	490 (33.9%)	37 (22.6%)		28 (24.6%)	9 (18.0%)	
Manganese (µg/l)			0.198			0.494
< 10.99	460 (31.9%)	63 (38.4%)		43 (37.7%)	20 (40.0%)	
10.99 - 14.29	480 (33.2%)	46 (28.0%)		33 (28.9%)	13 (26.0%)	

> 14.29	504 (34.9%)	55 (33.5%)		38 (33.3%)	17 (34.0%)	

Numbers did not equal 1909 women because of missing data on metals or HDP.
HDP= hypertensive disorders of pregnancy. GH overall=gestational hypertension with or without preeclampsia. GH= gestational hypertension without preeclampsia. DMA= dimethylarsinic acid.
[a] Chi-square test for the association between metals and Normotensive versus GH overall and Normotensive versus GH and preeclampsia.

Table S7. Unadjusted OR for the association between metal concentrations and HDP according to the type of specimen tested (blood versus urine) and trimester of measurement

Metals	n	GH overall OR (95% CI)[a]		n	GH OR (95% CI)[b]	Preeclampsia OR (95% CI)[b]
Blood first trimester				1779		
Arsenic (µg/l)	1779	0.96 (0.82, 1.12)		1779	0.85 (0.65, 1.11)	1.04 (0.91, 1.19)
Lead (µg/dl)	1779	0.62 (0.38, 0.99)*		1779	0.66 (0.38, 1.14)	0.53 (0.22, 1.27)
Cadmium (µg/l)	1779	0.89 (0.61, 1.29)		1779	0.68 (0.39, 1.19)	1.24 (0.78, 1.98)
Mercury (µg/l)	1779	0.78 (0.65, 0.95)**		1779	0.77 (0.61, 0.96)**	0.82 (0.60, 1.13)
Manganese (µg/l)	1779	0.96 (0.91, 1.01)		1779	0.97 (0.91, 1.03)	0.92 (0.83, 1.02)
Arsenic (µg/l)	1779			1779		
< 0.60		Reference			Reference	Reference
0.60 - 0.97		1.08 (0.75, 1.57)			1.03 (0.67, 1.58)	1.24 (0.63, 2.45)
> 0.97		0.96 (0.66, 1.41)			0.85 (0.54, 1.33)	1.29 (0.66, 2.51)
Lead (µg/dl)	1779			1779		
< 0.50		Reference			Reference	Reference
0.50 - 0.75		0.93 (0.66, 1.33)			1.15 (0.76, 1.73)	0.55 (0.29, 1.06)
> 0.75		0.56 (0.37, 0.83)**			0.54 (0.33, 0.89)**	0.58 (0.30, 1.12)
Cadmium (µg/l)	1779			1779		
< 0.16		Reference			Reference	Reference
0.16 - 0.27		1.13 (0.79, 1.62)			1.06 (0.70, 1.61)	1.32 (0.69, 2.50)
> 0.27		0.82 (0.55, 1.21)			0.74 (0.46, 1.17)	1.04 (0.52, 2.05)
Mercury (µg/l)	1779			1779		
< 0.42		Reference			Reference	Reference
0.42 - 1.06		0.97 (0.68, 1.38)			0.98 (0.64,1.49)	0.95 (0.52,1.75)
> 1.06		0.63 (0.42, 0.93)**			0.68 (0.43,1.07)	0.52 (0.26,1.07)
Manganese (µg/l)	1779			1779		
< 7.69		Reference			Reference	Reference
7.69 - 9.89		0.97 (0.67, 1.41)			0.95 (0.61, 1.49)	1.02 (0.55, 1.89)

> 9.89		0.67 (0.46, 0.99)*			0.77 (0.49, 1.21)	0.48 (0.23, 0.98)*
Urinary first trimester (µg As/l)						
DMA	1773	1.00 (0.97, 1.03)		1773	0.99 (0.95, 1.03)	1.01 (0.97, 1.06)
Arsenobetaine	1774	1.00 (1.00, 1.00)		1774	1.00 (0.99, 1.01)	1.00 (1.00, 1.00)
Inorganic Arsenic	1773	1.00 (0.98, 1.03)		1773	1.00 (0.97, 1.03)	1.01 (0.97, 1.05)
Total arsenic	1773	1.00 (1.00, 1.00)		1773	1.00 (0.99, 1.01)	1.00 (1.00, 1.00)
DMA	1773			1773		
< 1.50		Reference			Reference	Reference
1.50 - 3.67		0.98 (0.67, 1.42)			1.13 (0.73, 1.75)	0.67 (0.34, 1.34)
> 3.67		1.07 (0.74, 1.55)			1.06 (0.67, 1.65)	1.11 (0.60, 2.03)
Arsenobetaine	1774			1774		
< 0.38		Reference			Reference	Reference
0.38 - 1.95		1.17 (0.77, 1.79)			1.19 (0.72, 1.96)	1.14 (0.55, 2.37)
> 1.95		0.99 (0.70, 1.39)			1.00 (0.66, 1.50)	0.97 (0.55, 2.37)
Inorganic arsenic	1773			1773		
< 2.67		Reference			Reference	Reference
2.67 - 4.87		1.01 (0.69, 1.47)			1.17 (0.76, 1.85)	0.67 (0.34, 1.33)
> 4.87		1.11 (0.76, 1.60)			1.13 (0.72, 1.77)	1.07 (0.58, 1.97)
Total arsenic	1773			1773		
< 3.45		Reference			Reference	Reference
3.45 - 7.79		0.97 (0.67, 1.41)			1.28 (0.83, 1.98)	0.49 (0.24, 0.98)
> 7.79		0.95 (0.66, 1.38)			1.04 (0.66, 1.64)	0.81 (0.45, 1.48)
Blood third trimester						
Arsenic (µg/l)	1608	1.01 (0.90, 1.13)		1608	0.81 (0.62, 1.05)	1.11 (1.00, 1.23)
Lead (µg/dl)	1608	1.05 (0.70, 1.57)		1608	0.96 (0.59, 1.58)	1.24 (0.65, 2.37)
Cadmium (µg/l)	1608	0.80 (0.47, 1.38)		1608	0.60 (0.27, 1.34)	1.15 (0.58, 2.29)
Mercury (µg/l)	1608	0.60 (0.44, 0.80)**		1608	0.60 (0.42, 0.85)**	0.59 (0.35, 1.00)
Manganese (µg/l)	1608	0.98 (0.94, 1.02)		1608	0.99 (0.95, 1.04)	0.97 (0.90, 1.04)

Arsenic (µg/l) < 0.51 0.51 - 0.90 >0.90	1608	 Reference 1.03 (0.71, 1.51) 0.71 (0.47, 1.08)		1608	 Reference 0.82 (0.53, 1.28) 0.61 (0.38, 0.99)*	 Reference 1.80 (0.90, 3.59) 1.07 (0.50, 2.30)
Lead (µg/dl) < 0.46 0.46 - 0.68 > 0.68	1608	 Reference 1.05(0.71,1.56) 0.97(0.66,1.44)		1608	 Reference 1.17 (0.75, 1.84) 0.86 (0.53, 1.39)	 Reference 0.78 (0.37, 1.64) 1.23 (0.64, 2.35)
Cadmium (µg/l) < 0.16 0.16 - 0.25 > 0.25	1608	 Reference 0.91 (0.62, 1.35) 0.87 (0.59, 1.28)		1608	 Reference 0.87 (0.55, 1.37) 0.82 (0.52, 1.30)	 Reference 1.04 (0.52, 2.06) 0.99 (0.50, 1.96)
Mercury (µg/l) < 0.36 0.36 - 0.82 > 0.82	1608	 Reference 0.68 (0.47, 0.99)* 0.48 (0.32, 0.72)**		1608	 Reference 0.62 (0.40, 0.98)* 0.52 (0.32, 0.83)**	 Reference 0.82 (0.44, 1.55) 0.39 (0.18, 0.85)**
Manganese (µg/l) < 10.99 10.99 - 14.29 > 14.29	1608	 Reference 0.70 (0.47, 1.05) 0.80 (0.54, 1.17)		1608	 Reference 0.74 (0.46, 1.18) 0.81 (0.51, 1.27)	 Reference 0.62 (0.31, 1.27) 0.78 (0.40, 1.50)

Numbers did not equal 1909 women because of missing data on metals or HDP.
HDP= hypertensive disorders of pregnancy. GH overall=gestational hypertension with or without preeclampsia. GH= gestational hypertension without preeclampsia. CI= confidence interval. OR= odds ratio. DMA= dimethylarsinic acid.
[a] **Logistic regression model:** between each metal and GH overall versus Normotensive.
[b] **Multinomial regression model:** between each metal and GH and preeclampsia versus Normotensive.
* p <0.05
** p <0.03

Table S8. Associations between metal concentrations (continuous form) and HDP according to the type of specimen tested (blood versus urine) and trimester of measurement.

Metals	GH overall			GH	Preeclampsia
	n	Adjusted OR (95% CI)[a]	n	Adjusted OR (95% CI)[b]	Adjusted OR (95% CI)[b]
Blood first trimester					
Arsenic (µg/l)[c]	1621	0.98 (0.85, 1.14)	1778	0.85 (0.65, 1.11)	1.05 (0.92, 1.19)
Lead (µg/dl)[d]	1645	0.85 (0.51, 1.40)	1570	1.06 (0.60, 1.86)	0.52 (0.19, 1.46)
Cadmium (µg/l)e	1459	0.72 (0.35, 1.46)	1459	0.64 (0.27, 1.51)	0.93 (0.29, 3.01)
Mercury (µg/l)[f]	1593	0.92 (0.75, 1.12)	1591	0.84 (0.65, 1.09)	1.09 (0.81, 1.47)
Manganese (µg/l)[g]	1778	0.96 (0.91, 1.01)	1778	0.97 (0.91, 1.03)	0.92 (0.83, 1.02)
Urinary first trimester (µg As/l)					
DMA[h]	1772	0.98 (0.94, 1.02)	1772	0.96 (0.91, 1.02)	1.01 (0.96, 1.06)
Arsenobetaine[i]	1772	1.00 (1.00, 1.00)	1772	1.00 (0.99, 1.01)	1.00 (1.00, 1.00)
Inorganic arsenic[j]	1772	1.00 (0.97, 1.02)	1772	0.99 (0.96, 1.02)	1.01(0.97, 1.05)
Total arsenic[k]	1772	1.00 (1.00, 1.00)	1772	1.00 (0.99, 1.01)	1.00 (1.00, 1.00)
Blood third trimester					
Arsenic (µg/l)[l]	1607	1.01 (0.91, 1.13)	1447	0.77 (0.55, 1.08)	1.16 (1.03, 1.30)*
Lead (µg/dl)[m]	1490	1.37 (0.89, 2.12)	1474	1.54 (0.91, 2.61)	1.77 (0.88, 3.57)
Cadmium (µg/l)[n]	1410	0.80 (0.34, 1.90)	1386	0.74 (0.26, 2.05)	1.46 (0.40, 5.30)
Mercury (µg/l)[o]	1490	0.69 (0.50, 0.96)**	1374	0.67 (0.44, 1.04)	0.94 (0.55, 1.60)
Manganese (µg/l)[p]	1607	0.98 (0.94, 1.02)	1607	0.99 (0.95, 1.04)	0.97 (0.90, 1.04)

Numbers did not equal 1909 women because of missing data on metals, covariates or HDP on multivariate models.
HDP= hypertensive disorders of pregnancy. GH overall=gestational hypertension with or without preeclampsia. GH= gestational hypertension without preeclampsia. CI= confidence interval. OR= odds ratio. DMA= dimethylarsinic acid.

[a] **Logistic regression model (between each metal and GH overall versus Normotensive) adjusted for:**

[c] weight gain, maternal age

[d] BMI, mercury, maternal age

[e] education, fish consumption, maternal smoking, household income, BMI, weight gain, maternal age

[f] BMI, weight gain, maternal age

[g] maternal age
[h,i,j,k] specific gravity, maternal age
[l] maternal age
[m] BMI, mercury, maternal age
[n] education, maternal smoking, household income, weight gain, maternal age
[o] BMI, maternal age
[p] maternal age

[b] multinomial logistic regression model (between each metal and GH and preeclampsia versus Normotensive) adjusted for:

[c] maternal age
[d] BMI, household income, mercury, maternal age
[e] education, BMI, maternal smoking, household income, fish consumption, weight gain, maternal age
[f] education, BMI, weight gain, maternal age
[g] maternal age
[h,i,j,k] specific gravity, maternal age
[l] BMI, mercury, weight gain, maternal age
[m] ethnicity, education, BMI, maternal smoking, fish consumption, arsenic, mercury, manganese, maternal age
[n] education, BMI, maternal smoking, household income, weight gain, maternal age
[o] education, BMI, household income, fish consumption, weight gain, maternal age
[p] maternal age
[*] $p < 0.05$
[**] $p < 0.03$

Table S9**.** Crude OR for the associations between metal concentrations and HDP according to the trimester of measurements. All metals in the same model at each trimester.

Metals	GH overall	unadjusted OR (95% CI)[a]		GH unadjusted OR (95% CI)[b]	Preeclampsia unadjusted OR (95% CI)[b]
Blood first trimester	**n=1779**		**n=1779**		
Arsenic (µg/l)		1.02 (0.89, 1.15)		0.92 (0.72, 1.19)	1.06 (0.94, 1.20)
Lead (µg/dl)		0.72 (0.45, 1.16)		0.80 (0.46, 1.40)	0.56 (0.23, 1.38)
Cadmium (µg/l)		0.91 (0.63, 1.32)		0.69 (0.40, 1.20)	1.26 (0.78, 2.05)
Mercury (µg/l)		0.80 (0.66, 0.97)*		0.79 (0.63, 1.01)	0.85 (0.61, 1.17)
Manganese (µg/l)		0.96 (0.91, 1.02)		0.98 (0.91, 1.04)	0.93 (0.84, 1.03)
Arsenic (µg/l) < 0.60 0.60 - 0.97 > 0.97		 Reference 1.15 (0.79, 1.68) 1.19 (0.79, 1.79)		 Reference 1.07 (0.69, 1.68) 0.99 (0.61, 1.60)	 Reference 1.38 (0.70, 2.74) 1.81 (0.89, 3.70)
Lead (µg/dl) < 0.50 0.50 - 0.75 > 0.75		 Reference 0.96 (0.67, 1.37) 0.58 (0.38, 0.88)*		 Reference 1.20 (0.79, 1.83) 0.58 (0.35, 0.98)*	 Reference 0.54 (0.28, 1.05) 0.56 (0.28, 1.11)
Cadmium (µg/l) < 0.16 0.16 - 0.27 > 0.27		 Reference 1.25 (0.87, 1.81) 0.94 (0.63, 1.40)		 Reference 1.13 (0.74, 1.74) 0.81 (0.50, 1.30)	 Reference 1.60 (0.83, 3.08) 1.32 (0.65, 2.66)
Mercury (µg/l) < 0.42 0.42 - 1.06 > 1.06		 Reference 0.98 (0.68, 1.42) 0.65 (0.43, 0.99)*		 Reference 1.01 (0.66, 1.57) 0.73 (0.44, 1.19)	 Reference 0.91 (0.48, 1.71) 0.50 (0.23, 1.07)
Manganese (µg/l) < 7.69 7.69 - 9.89		 Reference 1.00 (0.68, 1.46)		 Reference 0.97 (0.62, 1.53)	 Reference 1.05 (0.56, 1.97)

> 9.89		0.70 (0.47, 1.04)		0.81 (0.51, 1.27)	0.49 (0.24, 1.00)
Blood third trimester	**n=1608**		**n=1608**		
Arsenic (µg/l)		1.07 (0.97, 1.18)		0.90 (0.70, 1.17)	1.15 (1.03, 1.29)
Lead (µg/dl)		1.37 (0.91, 2.07)		1.28 (0.78, 2.11)	1.63 (0.85, 3.09)
Cadmium (µg/l)		0.77 (0.45, 1.31)		0.58 (0.26, 1.27)	1.08 (0.55, 2.14)
Mercury (µg/l)		0.55 (0.40, 0.75)*		0.60 (0.41, 0.88)*	0.50 (0.28, 0.88)*
Manganese (µg/l)		0.99 (0.95, 1.03)		1.00 (0.95, 1.05)	0.97 (0.90, 1.04)
Arsenic (µg/l) < 0.51 0.51 - 0.90 > 0.90		 Reference 1.10 (0.75, 1.61) 0.88 (0.57, 1.36)		 Reference 0.87 (0.56, 1.37) 0.74 (0.44, 1.23)	 Reference 1.90 (0.95, 3.83) 1.40 (0.63, 3.12)
Lead (µg/dl) < 0.46 0.46 - 0.68 > 0.68		 Reference 1.20 (0.80, 1.80) 1.24 (0.82, 1.89)		 Reference 1.34 (0.84, 2.13) 1.10 (0.66, 1.83)	 Reference 0.89 (0.42, 1.91) 1.57 (0.78, 3.14)
Cadmium (µg/l) < 0.16 0.16 - 0.25 > 0.25		 Reference 0.95 (0.64, 1.42) 0.89 (0.59, 1.35)		 Reference 0.89 (0.56, 1.43) 0.84 (0.52, 1.37)	 Reference 1.11 (0.55, 2.25) 1.02 (0.49, 2.11)
Mercury (µg/l) < 0.36 0.36 - 0.82 > 0.82		 Reference 0.68 (0.46, 1.00) 0.49 (0.32. 0.76)*		 Reference 0.64 (0.40, 1.01) 0.57 (0.34, 0.95)*	 Reference 0.76 (0.40, 1.46) 0.34 (0.15, 0.78)*
Manganese (µg/l) < 10.99 10.99 - 14.29 > 14.29		 Reference 0.70 (0.47, 1.05) 0.83 (0.56, 1.23)		 Reference 0.74 (0.46, 1.20) 0.86 (0.54, 1.38)	 Reference 0.62 (0.30, 1.27) 0.76 (0.38, 1.50)

Numbers did not equal 1909 women because of missing data on metals or HDP.
HDP= hypertensive disorders of pregnancy. GH overall=gestational hypertension with or without preeclampsia. GH= gestational hypertension without preeclampsia. CI= confidence interval. OR= odds ratio.
[a]**Logistic regression:** between all metals and GH overall versus normotensive
[b]**Multinomial regression:** between all metals and GH and preeclampsia versus normotensive
*$p<0.05$

Table S10. Adjusted OR for the associations between metal concentrations and HDP according to the trimester of measurements. All metals in the same model at each trimester

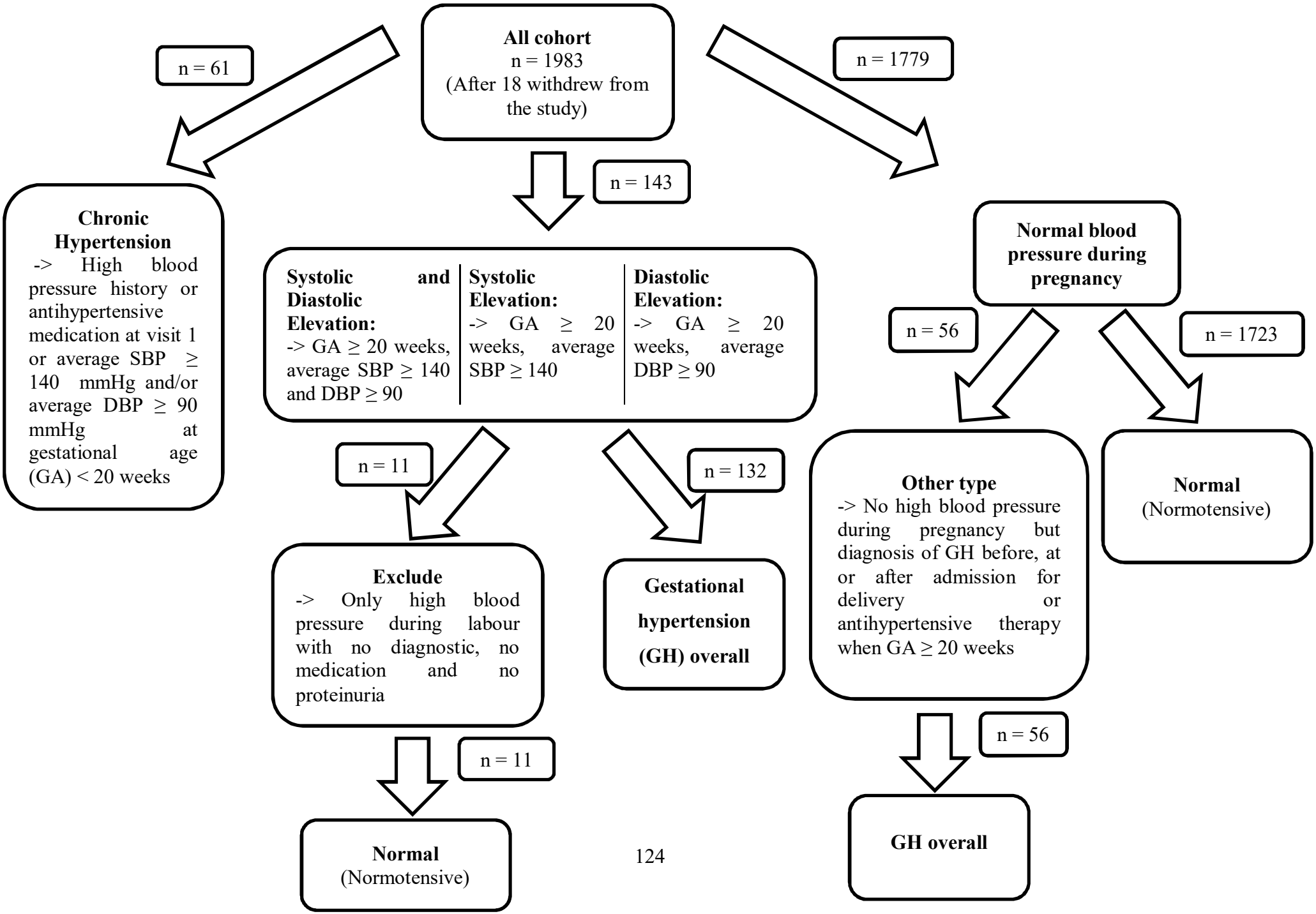
Metals	GH overall	Adjusted OR (95% CI)[a]		GH only Adjusted OR (95% CI)[b]	Preeclampsia Adjusted OR (95% CI)[b]
Blood first trimester[c]	**n=1519**		**n=1459**		
Arsenic (µg/l)		1.01 (0.88, 1.17)		0.93 (0.70, 1.22)	1.11 (0.96, 1.28)
Lead (µg/dl)		1.01 (0.60, 1.70)		1.20 (0.66, 2.17)	0.61 (0.20, 1.80)
Cadmium (µg/l)		0.86 (0.44, 1.67)		0.66 (0.28, 1.58)	0.91 (0.30, 2.74)
Mercury (µg/l)		0.94 (0.76, 1.15)		0.95 (0.73, 1.24)	1.12 (0.82, 1.54)
Manganese (µg/l)		0.93 (0.87, 0.99)*		0.93 (0.86, 1.00)	0.92 (0.82, 1.04)
Arsenic (µg/l)					
< 0.60		Reference		Reference	Reference
0.60 - 0.97		1.14 (0.74, 1.74)		1.07 (0.64, 1.77)	1.94 (0.83, 4.55)
> 0.97		1.16 (0.74, 1.84)		1.00 (0.57, 1.74)	2.75 (1.13, 6.73)*
Lead (µg/dl)					
< 0.50		Reference		Reference	Reference
0.50 - 0.75		1.15 (0.76, 1.73)		1.55 (0.94, 2.54)	0.52 (0.23, 1.15)
> 0.75		0.76 (0.47, 1.25)		0.82 (0.44, 1.52)	0.56 (0.24, 1.34)
Cadmium (µg/l)					
< 0.16		Reference		Reference	Reference
0.16 - 0.27		1.17 (0.77, 1.78)		1.07 (0.65, 1.76)	1.66 (0.76, 3.63)
> 0.27		0.85 (0.52, 1.38)		0.80 (0.45, 1.44)	1.04 (0.42, 2.53)
Mercury (µg/l)					
< 0.42		Reference		Reference	Reference
0.42 - 1.06		1.29 (0.84, 1.97)		1.35 (0.80, 2.26)	1.35 (0.60, 3.03)
> 1.06		0.92 (0.56, 1.52)		1.12 (0.60, 2.06)	1.05 (0.41, 2.70)
Manganese (µg/l)					
< 7.69		Reference		Reference	Reference
7.69 - 9.89		1.25 (0.82, 1.92)		0.94 (0.56, 1.57)	1.80 (0.83, 3.90)

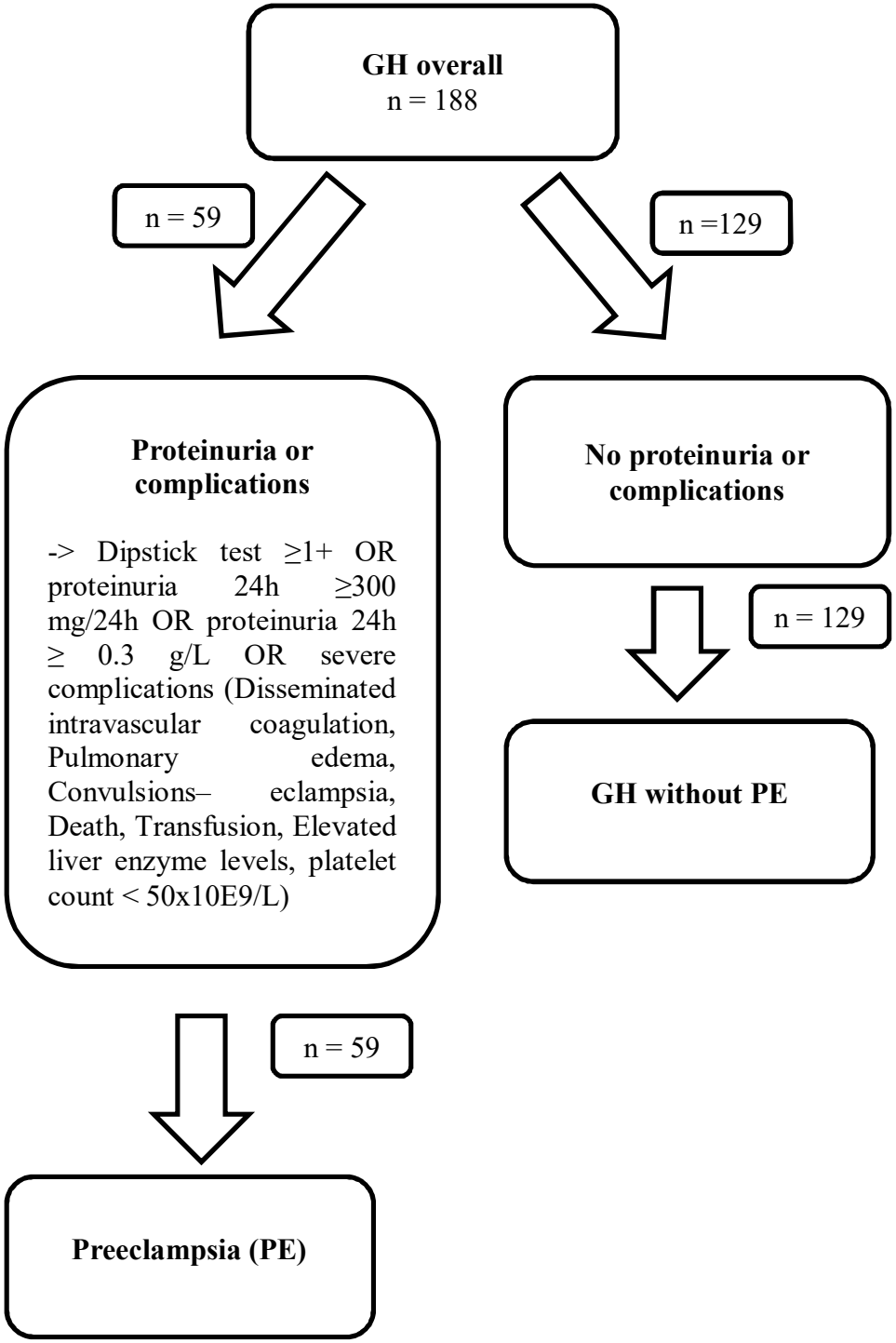
> 9.89		0.67 (0.42, 1.06)		0.68 (0.40, 1.15)	0.50 (0.20, 1.24)
Blood third trimester[d]	**n=1431**		**n=1374**		
Arsenic (µg/l)		1.04 (0.94, 1.17)		0.78 (0.55, 1.09)	1.13 (1.00, 1.28)
Lead (µg/dl)		1.46 (0.93, 2.28)		1.43 (0.83, 2.45)	1.62 (0.78, 3.35)
Cadmium (µg/l)		0.91 (0.41, 2.03)		0.77 (0.28, 2.14)	1.46 (0.39, 5.51)
Mercury (µg/l)		0.66 (0.47, 0.93)*		0.73 (0.47, 1.15)	0.79 (0.43, 1.46)
Manganese (µg/l)		0.98 (0.94, 1.02)		0.97 (0.92, 1.02)	0.97 (0.89, 1.05)
Arsenic (µg/l) < 0.51 0.51 - 0.90 > 0.90		 Reference 1.18 (0.78, 1.80) 0.80 (0.49, 1.29)		 Reference 0.97 (0.59, 1.59) 0.68 (0.38, 1.22)	 Reference 2.10 (0.93, 4.76) 1.43 (0.56, 3.62)
Lead (µg/dl) < 0.46 0.46 - 0.68 > 0.68		 Reference 1.22 (0.79, 1.90) 1.36 (0.85, 2.17)		 Reference 1.31 (0.78, 2.19) 1.28 (0.72, 2.27)	 Reference 0.89 (0.37, 2.11) 1.90 (0.83, 4.38)
Cadmium (µg/l) < 0.16 0.16 - 0.25 > 0.25		 Reference 1.10 (0.71, 1.71) 1.11 (0.69, 1.79)		 Reference 0.97 (0.57, 1.65) 0.99 (0.56, 1.75)	 Reference 1.27 (0.57, 2.84) 1.09 (0.46, 2.59)
Mercury (µg/l) < 0.36 0.36 - 0.82 > 0.82		 Reference 0.77 (0.50, 1.18) 0.66 (0.40, 1.09)		 Reference 0.78 (0.46, 1.31) 0.74 (0.39, 1.38)	 Reference 0.96 (0.44, 2.09) 0.65 (0.24, 1.77)
Manganese (µg/l) < 10.99 10.99 - 14.29 > 14.29		 Reference 0.76 (0.49, 1.18) 0.73 (0.47, 1.13)		 Reference 0.83 (0.49, 1.41) 0.71 (0.42, 1.22)	 Reference 0.73 (0.32, 1.66) 0.68 (0.31, 1.50)

Numbers did not equal 1909 women because of missing data on metals, covariates or HDP on multivariate models.
HDP= hypertensive disorders of pregnancy. GH overall=gestational hypertension with or without preeclampsia. GH= gestational hypertension without preeclampsia. CI= confidence interval. OR= odds ratio.
[a]**Logistic regression:** between all metals and GH overall versus normotensive
[c]adjusted for maternal age, education, BMI, maternal smoking, household income, weight gain
[d]adjusted for maternal age, BMI, maternal smoking, household income
[b]**Multinomial regression:** between all metals and GH and preeclampsia versus normotensive
[c]adjusted for maternal age, BMI, education, household income, fish consumption, weight gain, maternal smoking
[d]adjusted for maternal age, BMI, maternal smoking, fish consumption, education, weight gain, household income
[*]$p<0.05$

Figure legends

Figure S1. An algorithm summarizing the steps in the diagnosis of Hypertensive disorders of pregnancy (HDP)


All cohort
n = 1983
(After 18 withdrew from the study)
n = 61
n = 1779
n = 143
Chronic Hypertension
-> High blood pressure history or antihypertensive medication at visit 1 or average SBP ≥ 140 mmHg and/or average DBP ≥ 90 mmHg at gestational age (GA) < 20 weeks
Systolic and Diastolic Elevation:
-> GA ≥ 20 weeks, average SBP ≥ 140 and DBP ≥ 90
Systolic Elevation:
-> GA ≥ 20 weeks, average SBP ≥ 140
Diastolic Elevation:
-> GA ≥ 20 weeks, average DBP ≥ 90
Normal blood pressure during pregnancy
n = 56
n = 1723
n = 11
n = 132
Exclude
-> Only high blood pressure during labour with no diagnostic, no medication and no proteinuria
Gestational hypertension (GH) overall
Other type
-> No high blood pressure during pregnancy but diagnosis of GH before, at or after admission for delivery or antihypertensive therapy when GA ≥ 20 weeks
Normal
(Normotensive)
n = 11
n = 56
Normal
(Normotensive)
GH overall


GH overall
n = 188
n = 59
n =129
Proteinuria or complications
-> Dipstick test ≥1+ OR proteinuria 24h ≥300 mg/24h OR proteinuria 24h ≥ 0.3 g/L OR severe complications (Disseminated intravascular coagulation, Pulmonary edema, Convulsions– eclampsia, Death, Transfusion, Elevated liver enzyme levels, platelet count < 50x10E9/L)
No proteinuria or complications
n = 129
GH without PE
n = 59
Preeclampsia (PE)

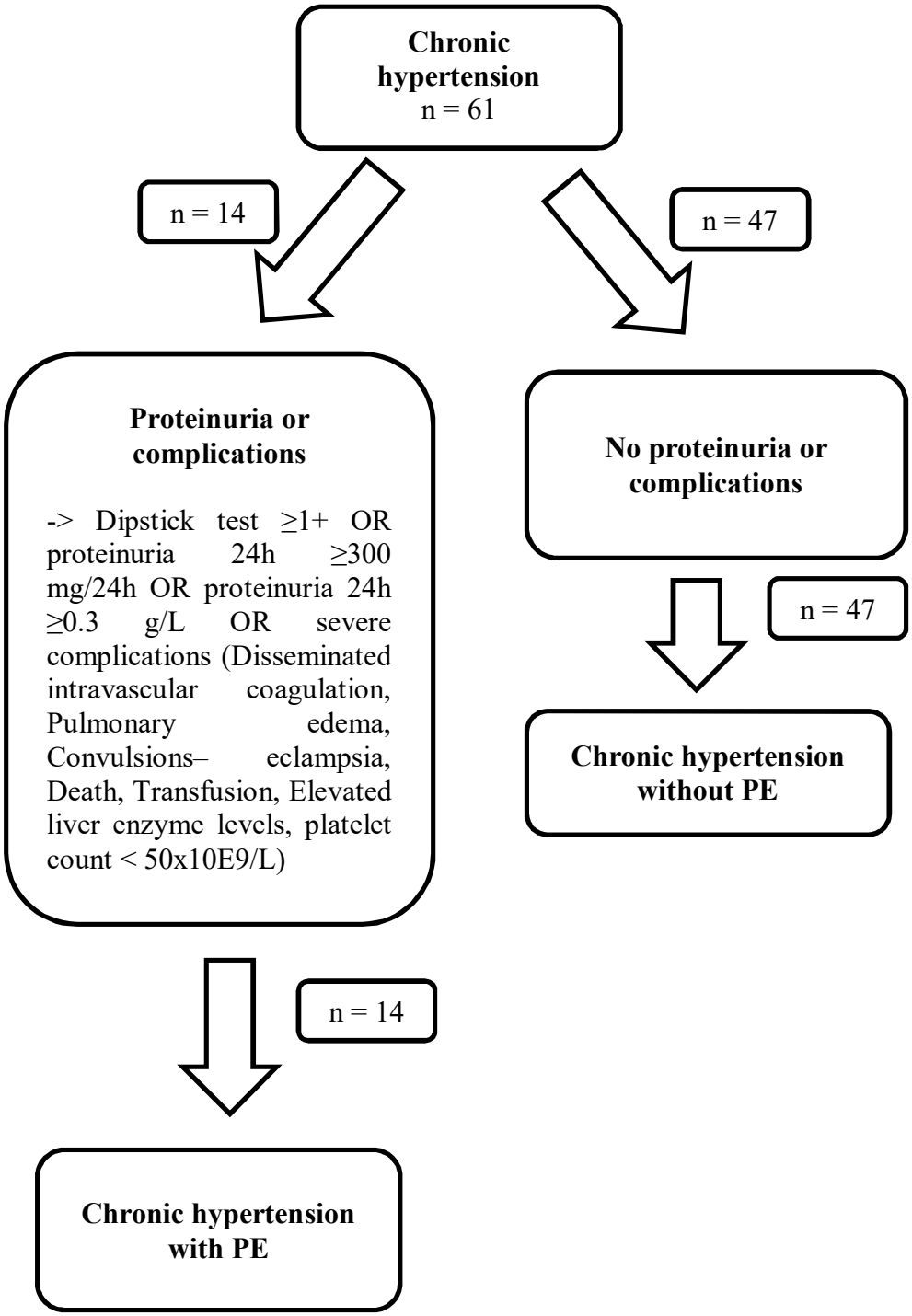
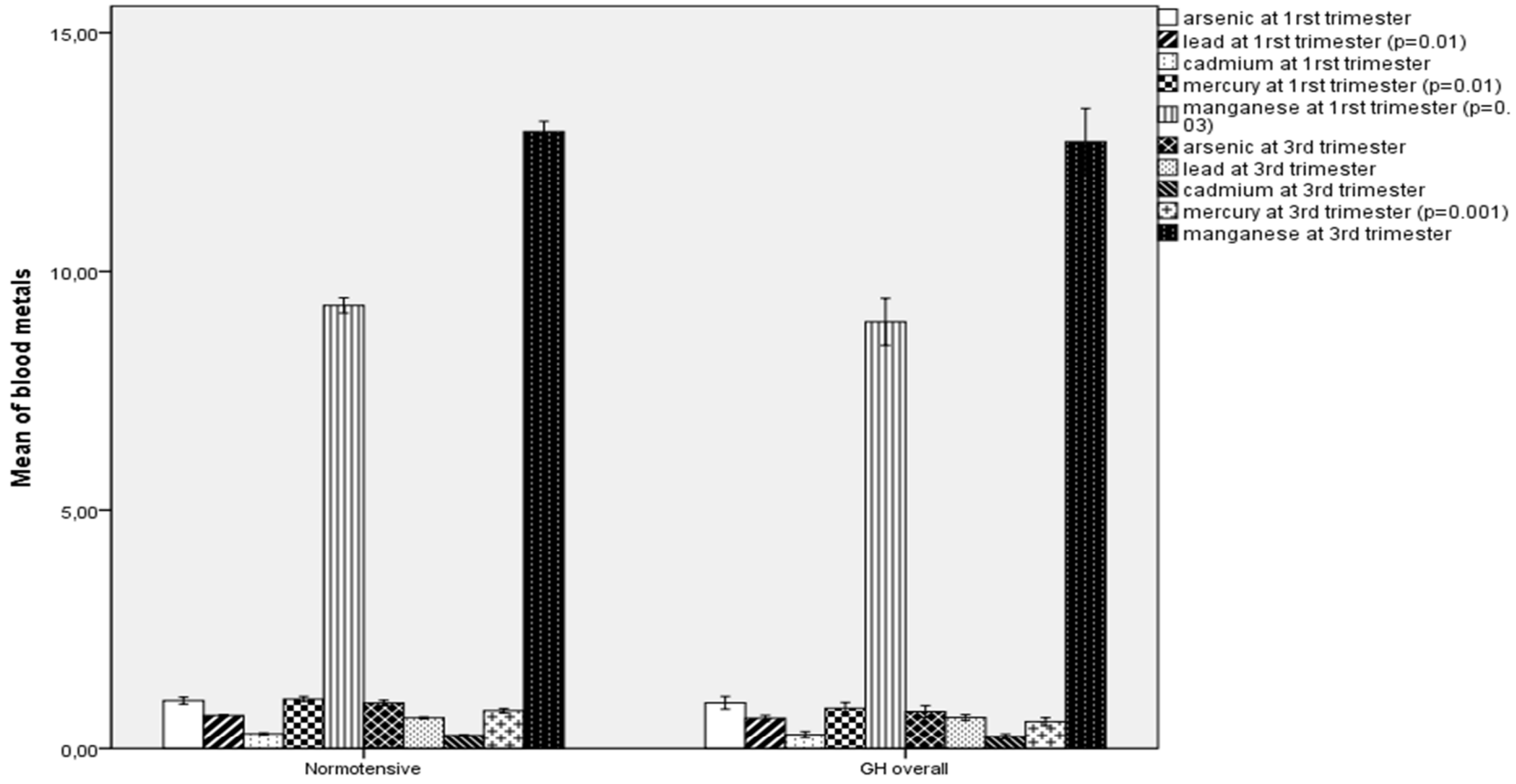
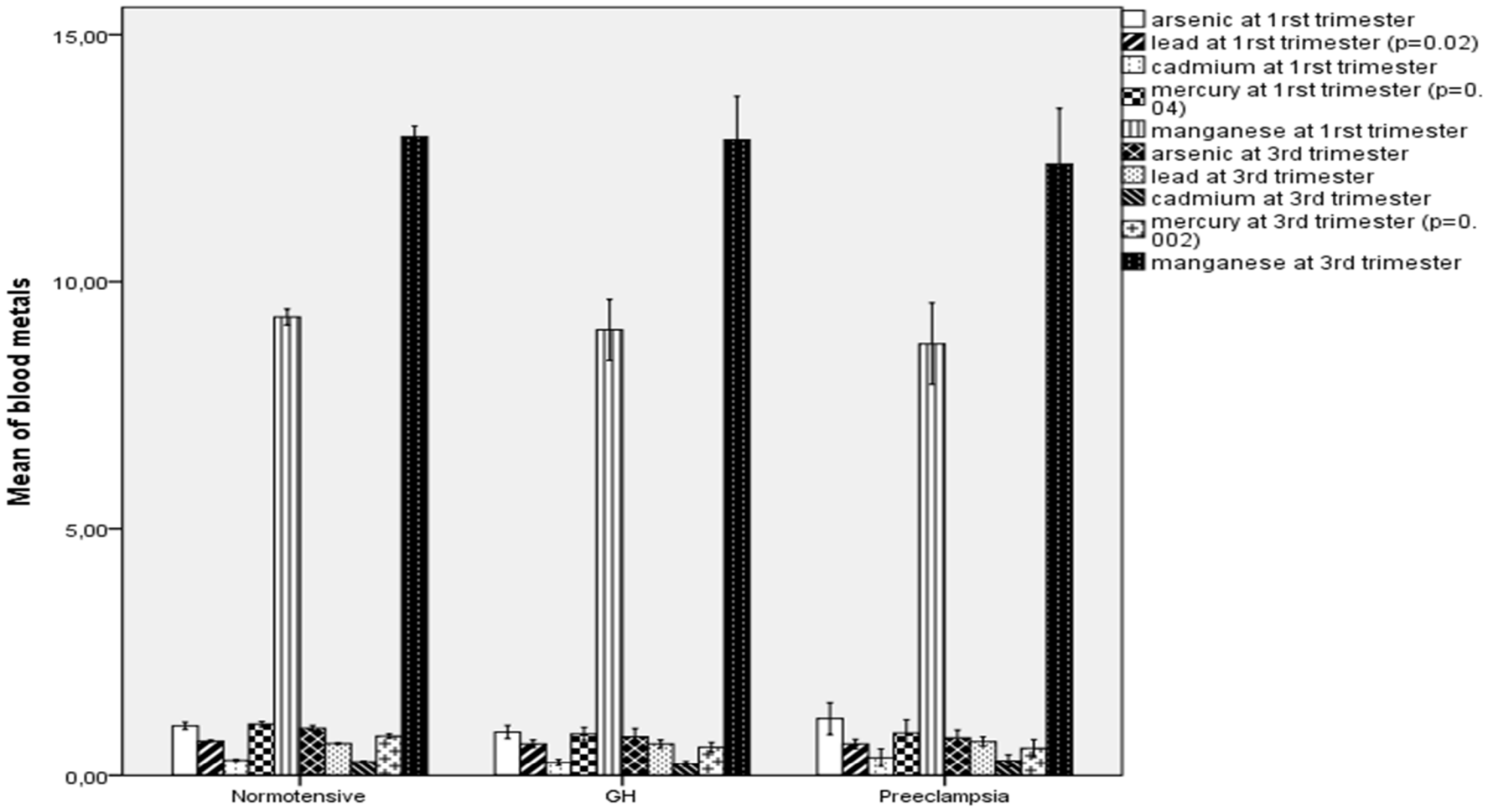

Chronic hypertension
n = 61
n = 14
n = 47
Proteinuria or complications
-> Dipstick test ≥1+ OR proteinuria 24h ≥300 mg/24h OR proteinuria 24h ≥0.3 g/L OR severe complications (Disseminated intravascular coagulation, Pulmonary edema, Convulsions– eclampsia, Death, Transfusion, Elevated liver enzyme levels, platelet count < 50x10E9/L)
No proteinuria or complications
n = 47
Chronic hypertension without PE
n = 14
Chronic hypertension with PE

Figure S2. Concentrations of blood metals (µg/l) according to GH overall or HDP status

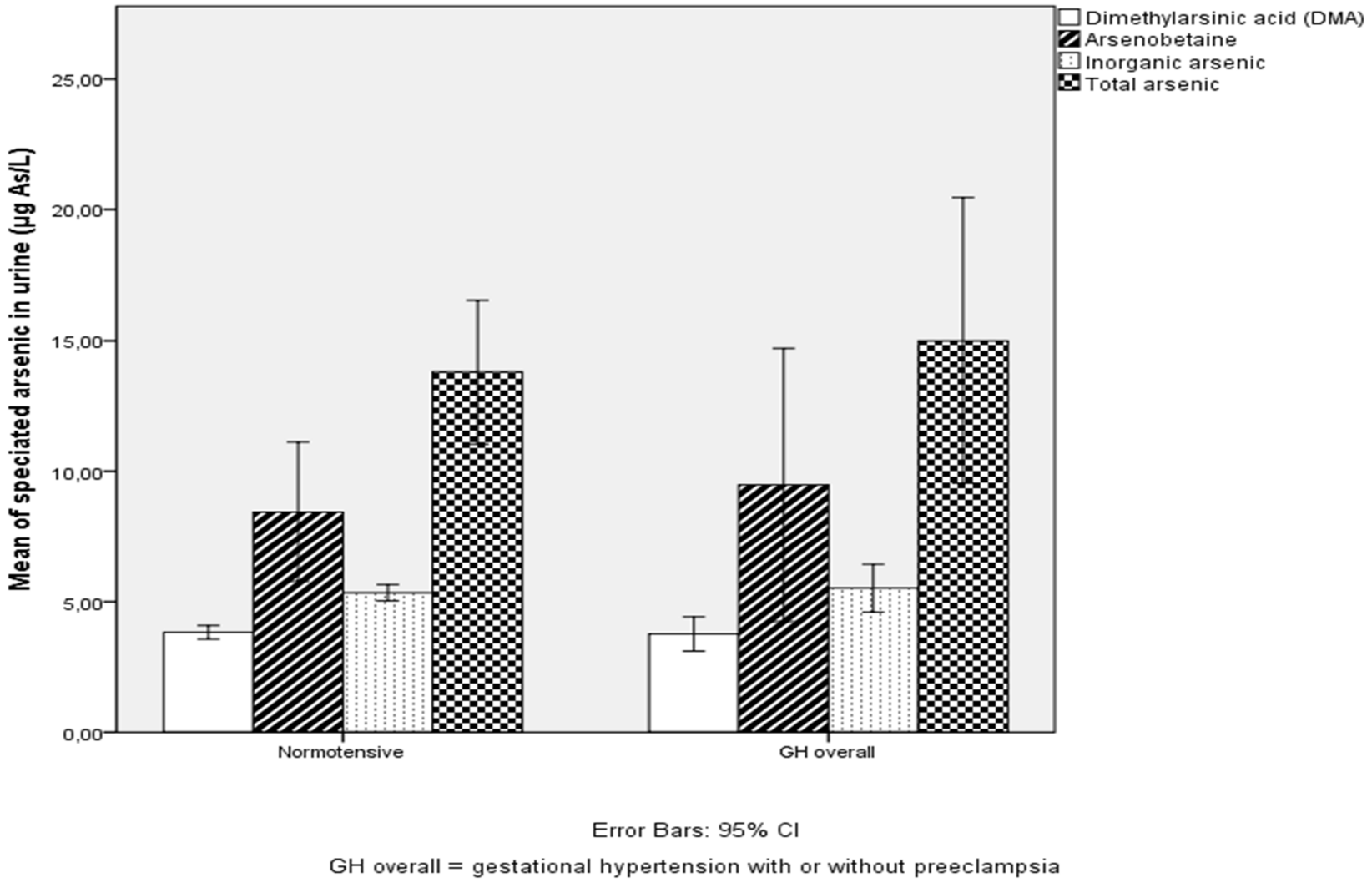


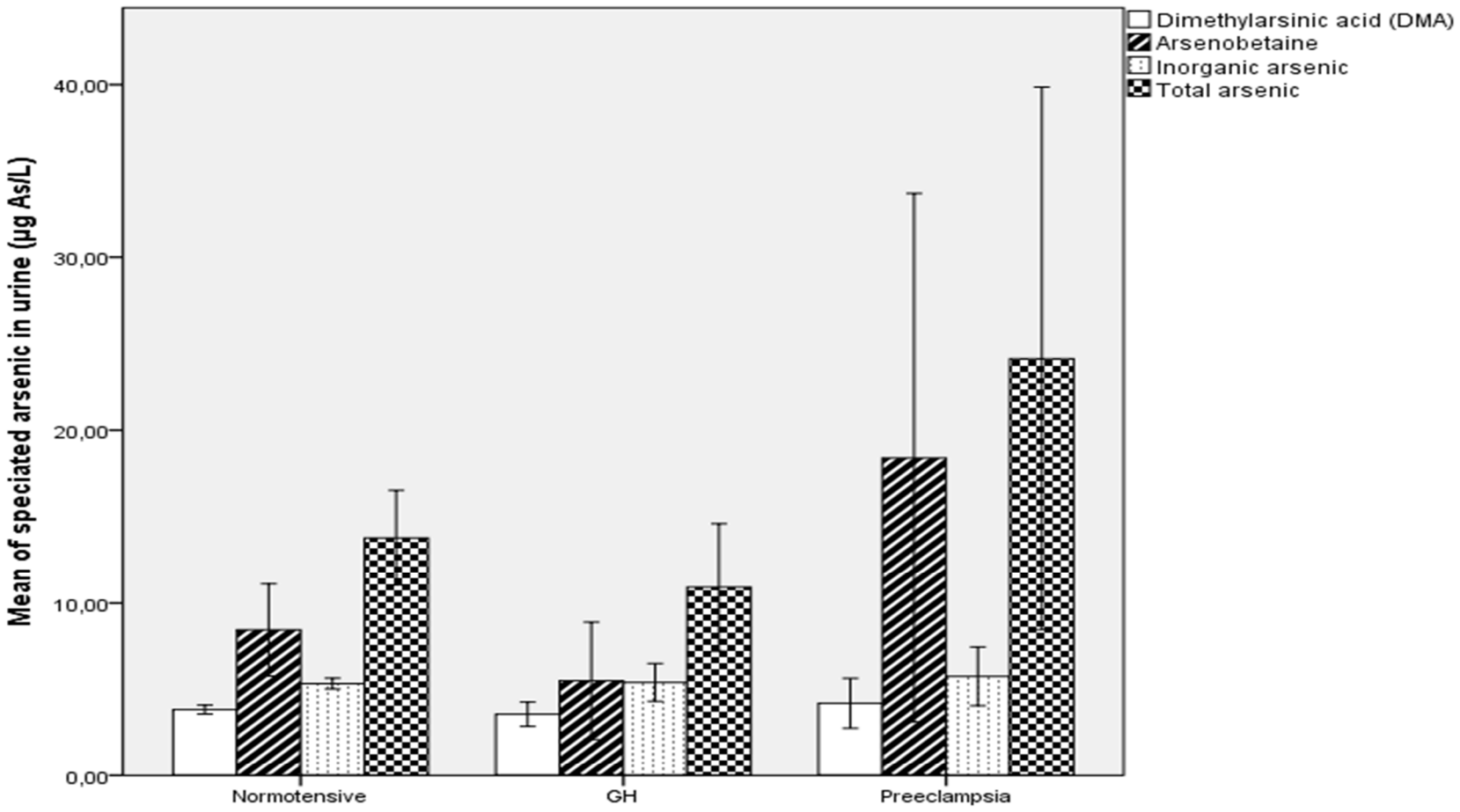


Error Bars: 95% CI

GH = gestational hypertension without preeclampsia

Figure S3. Concentrations of speciated arsenic measured in urine according to GH overall or HDP status





Error Bars: 95% CI

GH = gestational hypertension without preeclampsia

Chapitre 6 - Dental amalgams and risk of gestational hypertension in the MIREC Study (Article 2)

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Statut : le manuscrit est en correction à Santé Canada. L'article sera soumis à New England Journal of Medecine.

Contribution des auteurs :

- Louopou Rosalie Camara a constitué la base de données, elle a fait toutes les analyses statistiques ainsi que la rédaction du manuscrit sous la supervision de Dre Helen Trottier et de Dr William D. Fraser.

- Les coauteurs : Dre Helen Trottier a participé aux analyses et à la rédaction du manuscrit. Dr William D. Fraser est l'un des principaux chercheurs de l'étude MIREC. Ils ont participé à la conception et au design de l'étude, à sa conduite, à la rédaction du manuscrit et aux interprétations des données.

Abstract

Background: The potential association between the presence or replacement of dental amalgams and gestational hypertension (GH) is unclear.

Objective: To assess the association between the presence and replacement of dental amalgams and the risk of GH in a Canadian prospective cohort study of pregnant women.

Methods: We assessed dental amalgam status (presence or replacement), blood mercury concentrations, and measured blood pressure in 1909 pregnant women recruited in 10 Canadian cities between 2008 and 2011 as part of the Maternal-Infant Research on Environmental Chemicals (MIREC) study. Blood pressure was assessed in each trimester of pregnancy and mercury concentrations in 1st and 3rd trimesters. Logistic regression analysis was performed to estimate the adjusted odds ratios (aORs) and 95% confidence intervals (CI) for the associations between dental amalgam status and GH. The associations between concurrent measures of dental amalgam status and GH were assessing through logistic generalized estimating equations.

Results: Dental amalgam status was weakly correlated with mercury concentrations and there was no evidence of an association with GH (adjusted OR (aOR) = 1.31 (95%CI: 0.92, 1.85) and aOR = 1.32 (95% CI: 0.86, 2.04) for women having 1-4 or ≥ 5 dental amalgams, respectively, compared to women without amalgam reported at first trimester). Dental amalgam replacement reported in the first or third trimester was not associated with GH (aOR = 0.75 (0.40, 1.42) and aOR = 0.73 (0.39, 1.34), respectively).

Conclusion: We found weak correlations between dental amalgams and blood mercury among pregnant women. Moreover, the presence of dental amalgams or their replacement was not associated with GH. Further studies are required to confirm these results.

Introduction

Gestational hypertension with or without preeclampsia (GH)[1] occurred in 10% of pregnancies.[2] Its etiology remains uncertain.[3] Exposure to metals has been associated with GH [4], however findings are not consistent.[5,6] Dental amalgams contain metals (approximately 50% of elemental mercury).[7] Previous studies have suggested an association between mercury exposure and hypertension [4,8,9] which might be explained through increased oxidative stress, reduced nitric oxide bioavailability, endothelial dysfunction and vasoconstriction.[8,9] To date, no one has explored the possible association between dental amalgam status (presence or replacement) and the risk of GH.

Dental amalgams are restorative materials that have been used for more than 150 years.[7] The average life of dental amalgams is 8.3 years.[10] Fracture is the major cause of their failure.[10,11] Dental amalgams can release small amounts of elementary mercury into the body [12,13,14] for years.[15] Elementary mercury can be transformed into methylmercury [16] which is more toxic.[17,18] Elemental mercury from ingestion is poorly absorbed with a bioavailability of less than 0.01% but it can increase during a gastrointestinal tract defect.[19] Mercury has no known physiological function in the body [16] and adverse effects may be offset by consumption of fish containing omega-3 fatty acids.[9]

Mercury has been found in the blood of pregnant women at variable levels.[20,21] The variability in blood mercury attributed to dental amalgams in pregnant women has been estimated at 6.47% compared to 8.75% for seafood consumption.[22] Moreover, dental amalgam removal and replacement may be associated with higher prenatal exposure to mercury compared to new amalgam emplacement.[23] Some studies have suggested that dental amalgams can adversely

impact health[24-29] including blood pressure.[30] Thus, our objective is to explore the potential association between dental amalgam status (presence or replacement) and the risk of GH.

Methods

Study design and population

Our analysis is based on data from the MIREC Study which is a prospective cohort study of 2001 pregnant women recruited between 2008-2011 in 10 Canadian cities (Vancouver, Edmonton, Winnipeg, Sudbury, Ottawa, Kingston, Hamilton, Toronto, Montreal, and Halifax). Full details of the MIREC study population have been published previously.[31] Briefly, generally healthy women were recruited in their first trimester of pregnancy and followed until after delivery. Maternal blood collected during the first and third trimesters were analyzed for total mercury. Blood pressure was measured during each trimester of pregnancy as well as after hospital admission for delivery. In addition, women completed a staff-administrated questionnaire at each visit in order to collect information on socio-demographic, exposure characteristics and other variables. Clinical data throughout pregnancy were abstracted from the medical charts. Of the 2001 consenting pregnant women, 18 withdrew from the study and requested that their data be destroyed. After recruitment, 74 women were excluded due to miscarriage or stillbirth, leaving 1909 women in the cohort. The study was approved by Health Canada's Research Ethics Board and the Research Ethics Committee of Sainte-Justine University Hospital in Montreal, Quebec (Canada) as well as in all MIREC affiliated recruitment centers. All participants signed consent forms.

Dental amalgam information

Information on dental amalgams was obtained as part of a larger questionnaire administered at scheduled first and third trimester visits. More specifically, women were asked at each visit: "currently, how many mercury-silver dental fillings do you have?" In addition, at the first trimester visit, they were asked: "within the past 12 months, have you had any mercury-silver (also known as amalgam) dental fillings replaced?" and at the third trimester visit: "since visit 1, have you had any mercury-silver (also known as amalgam) dental fillings replaced?" The specific composition of the dental amalgams was not available.

Total Mercury

Total mercury concentrations were measured in first and third trimester maternal blood using a single-quadrupole inductively coupled plasma mass spectrometry (ICP-MS) Elan DRC-II system (Perkin Elmer, Norwalk CT, USA) with a limit of detection (LOD) of 0.1204 μg/l (0.6 nmol/l). The analysis was performed at the Centre de Toxicologie du Québec, Institut National de Santé Publique du Québec, Quebec, Canada. Concentrations below the LOD were assigned a value equivalent to half the LOD. Less than 12% of participants had blood mercury concentrations below the LOD.

Blood pressure and diagnosis of GH

Blood pressure was assessed in a sitting position by the study staff using a sphygmomanometer at each clinic visit. Two measures of blood pressure were taken about 1 minute apart and averaged for each visit. The Korotkoff phase V (disappearance) was used for diastolic blood pressure (DBP) measurement. The diagnostic criteria for GH are based on the Society of Obstetricians and Gynaecologists of Canada guidelines.[1] According to this guideline, the appearance of hypertension at ≥ 20 weeks of gestation based on the average of two measurements of systolic

blood pressure (SBP) ≥ 140 mmHg and/or DBP ≥ 90 mmHg, taken at least 1 minute apart in MIREC study, defines GH which include women with or without preeclampsia.[1] In this study GH is measured between week 20 of pregnancy and the day of discharge from the hospital after delivery. Gestational age in weeks was based on last menstrual period and/or early ultrasound result.

Covariates

Potential covariates were derived from questionnaires administred in each trimester as well as from medical chart reviews. The following variables were analyzed as potential covariates: maternal age at delivery in years (continuous form), parity (multiparous, nulliparous), ethnicity (caucasian, non-caucasian), body mass index before pregnancy (BMI) (weight in kg divided by height squared in meters), weight gain during pregnancy (difference between last weight measured prior to delivery and weight measured at first trimester visit), both as continuous variables, education (graduate university, undergraduate university, college, less college), household income ($ < 65,000, 65,000 – 90,000, > 90,000), maternal smoking (no, yes), and fish consumption (relative total quantity serving size per day, calculated from the food frequency questionnaire by Morisset et al. (2016)), coffee intake (no, yes), multiple child pregnancy (no, yes), women with auto-immune disease (no, yes). Because of the high proportion of missing data (35%) concerning gestational diabetes status, this covariate was not considered as a potential confounder.

Statistical analysis

Descriptive statistics for maternal characteristics were estimated according to dental amalgam status (number of dental amalgams reported at first or third trimester visits, replacement of dental

amalgams within the past 12 months reported at first trimester visit, and replacement during pregnancy reported at third trimester visit). Comparisons for continuous variables were conducted with Student's t-test, or univariate anova, Mann-Whitney or Kruskal-Wallis tests and for categorical variables using chi-square tests. Medians (interquartile range (IQR)) and geometric means (GM) of mercury were determined according to dental amalgam status and were compared using Mann-Whitney or Kruskal-Wallis. Finally, Spearman correlation was used to test the correlations between blood mercury, dental amalgam status and fish consumption.

Logistic regression models were used to analyze the association between dental amalgam status and GH (yes or no). Presence of dental amalgams was assessed according to their number (categorized as 0, 1-4 and ≥ 5) and the time at which they were reported (first or third trimester visit). We also analyzed the impact of replacements according to data reported at first and third trimester visits, i.e. replacement within 12 months prior to first trimester visit and replacement during pregnancy (reported at third trimester visit). Crude and adjusted odds ratios (ORs) and 95% confident intervals (CI) were estimated. Adjustment was made for covariates selected either *a priori* on the basis of evidence of potential confounding from the literature (maternal age) or empirically. We examined the above-mentionned covariates separately for a potential confounding bias using change-in-estimate method. Those that changed the ORs of the association between dental amalgam status and GH by ±10% were considered as confounders and included in the multivariable models. In order to optimize power in multivariate models, covariates were considered in a continuous form when possible and missing data were ignored. This may have reduced the number of women in certain multivariate models but as missing data were rare and considered "*missing completely at random* (MCAR)", it did not have an important

impact on results. We also did two sensitivity analyses: 1) including or or excluding women who reported dental amalgam in the first trimester but had missing data in the third trimester and 2) including or excluding fish consumption in the model. P-values of <0.05 were considered to indicate statistical significance.

We also used logistic generalized estimating equations to explore the relationship between dental amalgam status (repeated variable measured at first and third trimester) and GH (not repeated binary variable and measured after first trimester at ≥ 20 weeks). Logistic generalized estimating equations take into account the clustering within each individual caused by the repeated-measurements design. Models incorporated a first-order autoregressive correlation pattern for the repeated events. All statistical analyses were performed with IBM SPSS Statistics Version 22, SAS 9.4 for Windows and R for Windows 3.2.2.

Results

A total of 1630 (85.4%) study participants were categorized as normotensive and 187 (9.8%) as GH. Women with chronic hypertension (n = 58 (3%)) or without data on hypertension status (n = 34 (1.8%)) were treated as missing data with respect to GH. In Table 1, the majority of the participants were Caucasian, university educated, multiparous, non-smokers and had a household income more than $90,000. Mean maternal age was positively associated with number of dental amalgams and replacement status. BMI and maternal weight gain were significantly higher in women who reported dental amalgams compared to those without amalgams. Fish consumption was statistically associated with the replacement of dental amalgams.

As Table 2 reflects, blood mercury concentrations were higher in women who reported dental amalgams and also among those with any replacements within 12 months prior to the first

trimester visit or replacement during pregnancy (all p-values <0.05). Spearman correlations between blood mercury and dental amalgams were not high. First trimester mercury concentrations were significantly (but weakly) correlated with the presence of dental amalgams reported as assessed at both the first and third trimester visits (Spearman's rho (r) = 0.156, p <0.001 and r = 0.153, p <0.001; respectively) and to a lesser extent with the report of any replacements within the past 12 months (r = 0.069, p = 0.003) or replacement during pregnancy (reported in the 3rd trimester visit) (r = 0.064, p = 0.006) (data not shown). Similar results were found for the blood mercury concentrations measured on specimens obtained at the third trimester visit (r = 0.176, p <0.001; r = 0.162, p <0.001; r = 0.087, p <0.001 and r = 0.075, p = 0.002; respectively). Mercury concentrations measured on maternal blood taken at first and third trimester visits were strongly correlated (r = 0.758, p <0.001). Fish consumption was moderately correlated with mercury concentrations (r = 0.460, p <0.001 at first trimester visit and r = 0.488, p <0.001 at third trimester visit) and weakly with any replacements 12 months prior to the first visit (r = 0.051, p = 0.029) (data not shown).

In Table 3, dental amalgam status was not statistically associated with GH either in unadjusted or adjusted models. The adjusted ORs (aORs) (95% CI) for the outcome of GH according to the presence of dental amalgam reported in the first trimester visit were 1.31 (0.92, 1.85) and 1.32 (0.86, 2.04) for women with 1-4 amalgams and with ≥ 5 amalgams, respectively, compared to those without amalgam. Findings were similar for the presence of amalgams reported at the third trimester visit. Similarly, no statistically significant associations were found for amalgam dental replacement. The aORs were 0.75; 95% CI: 0.40, 1.42, and 0.73; 95% CI:

0.39, 1.34, respectively for replacement 12-month prior first trimester visit and replacement during pregnancy.

Two sensitivity analyses were conducted: 1) excluding or not women who reported dental amalgam in the first trimester but had missing data in the third trimester and 2) considering fish consumption in the model. The first sensitivity analysis showed minimal changes in the results with the presence of dental amalgam (aOR = 1.11; 95% CI: 0.74, 1.66 and aOR = 1.20; 95% CI: 0.73, 1.96) for women with 1-4 and with ≥ 5 amalgams, respectively, compared to those without amalgam as reference) (data not shown). In addition, models considering fish consumption did not substantially change the associations.

The results for the logistic GEE models are presented in table 4. The presence of dental amalgam or any replacements (the first and third trimester measure taken together) were not statistically associated with the risk of GH (aOR = 1.02; 95% CI: 0.99, 1.05) and aOR = 0.99; 95% CI: 0.99, 1.00) respectively).

Discussion

No statistically significant associations between dental amalgam status and risk of GH were found in our study although blood mercury concentrations were correlated to the number of amalgams or with amalgam replacement.

To our knowledge, no other study has analyzed the association between dental amalgam and hypertensive disorders during pregnancy or blood pressure in pregnant women. Two cross-sectional study, one among 263 pregnant women (GM = 0.13 µg/l (0.10, 0.17)[32] and one in 262 dental professionnals (mean = 0.94 µg/l)[33] found a negative association between inorganic mercury SBP althoug no association was found for DBP. Studies in the general population are

not consistent on the association between dental amalgams and cardiovascular disease. A prospective study with 1462 Sweden women reported no association between dental amalgams and cardiovascular diseases.[34,35] In contrast, another study[30] showed an association between mercury from dental amalgam and cardiovascular disorders including high blood pressure.

In our study, mercury concentrations were weakly correlated with the presence or replacement of dental amalgam. Some studies have demonstrated a moderate or high correlation between mercury and dental amalgams using different types of sample such as cord blood (r = 0.46)[21], saliva (r = 0.93)[36,37] or hair (r = 0.92).[37]

It is interesting to highlight that the concentrations of blood mercury measured in the first trimester (0.51, 0.64, and 0.86 μg/l for women with 0, 1-4 and ≥ 5 dental amalgams, respectively were higher than the concentrations of mercury measured at third trimester (0.40, 0.51 and 0.71 μg/l, respectively). This difference may be explained by the capacity of mercury to bond to hemoglobin[38], the concentration of which decreases over the course of pregnancy.[39] Hemoglobin levels are higher in cord than maternal blood[40] which suggest that mercury concentration is higher in cord than in maternal blood as reported by our previous study.[41] Similar results were found by others authors.[42-44]

Strengths and limitations of the study

To our knowledge this is the first study to examine the association between dental amalgams and risk of GH. Laboratory measurements were performed in a national reference laboratory. The prospective cohort design increases the validity of study findings. Our study has a large sample size (n = 1909) but the proportion of GH (9.8%) may be not sufficient and may have limited the

power to detect an association with dental amalgam status in this group, if such an association was present.

Conclusion

The number or replacement of dental amalgams, although associated with blood mercury concentration, was not significantly associated with an increased risk of gestational hypertension in our study population. Additional studies in pregnant women may help to confirm these results.

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Competing financial interests declaration

The authors declare no actual or potential competing financial interests.

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Table 1. Participant characteristics according to dental amalgam status reported at recruitment.

Characteristics	**Number of dental amalgam reported at 1st trimester visit**			**Replacement within 12 months prior to 1st trimester visit**	
	0	**1-4**	**≥ 5**	**No**	**Yes**
Maternal age (years), mean ± SD	31.95 ± 5.04*	33.33 ± 4.89*	34.50 ± 5.07*	32.76 ± 5.06*	34.46 ± 4.96*
Weight gain (kg), mean±SD	15.16± 6.37*	15.05± 6.27*	13.91± 6.31*	14.99± 6.30	14.34± 6.44
BMI, median (IQR)	23.30 (20.87, 26.37)*	23.39 (21.26, 27.11)*	24.32 (21.69, 28.32)*	23.54 (21.16, 26.68)	23.58 (21.66, 27.10)
Ethnicity, n (%)[b]					
Caucasian	789 (86.6%)	530 (84.7%)	280 (87.5%)	1512 (86.4%)	128 (81.5%)
Non-caucasian	122 (13.4%)	96 (15.3%)	40 (12.5%)	238 (13.6%)	29 (18.5%)
Education					
Graduate University	240 (26.3%)	157 (25.2%)	82 (25.6%)	449 (25.7%)	46 (29.5%)
Undergraduate University	333 (36.6%)	231 (37.0%)	113 (35.3%)	640 (36.6%)	56 (35.9%)
College	239 (26.2%)	179 (28.7%)	84 (26.3%)	470 (26.9%)	42 (26.9%)
Less college	99 (10.9%)	57 (9.1%)	41 (12.8%)	190 (10.9%)	12 (7.7%)
Household income ($CAN)					
< 65,000	266 (30.6%)	177 (29.6%)	95 (30.6%)	509 (30.5%)	42 (28.2%)
65,000 – 90,000	256 (29.5%)	169 (28.3%)	94 (30.3%)	485 (29.1%)	44 (29.5%)
> 90,000	346 (39.9%)	252 (42.1%)	121 (39.0%)	675 (40.4%)	63 (42.3%)
Parity					
Multiparous	511 (56.1%)	342 (54.8%)	199 (62.2%)	984 (56.3%)	92 (59.0%)
Nulliparous	400 (43.9%)	282 (45.2%)	121 (37.8%)	765 (43.7%)	64 (41.0%)
Maternal smoking					
No	855 (93.9%)	589 (94.1%)	295 (92.2%)	1637 (93.5%)	151 (96.2%)
Yes	56 (6.1%)	37 (5.9%)	25 (7.8%)	113 (6.5%)	6 (3.8%)
Fish consumption (serving/day), median (IQR)	0.10 (0.03, 0.14)	0.09 (0.02, 0.14)	0.13 (0.03, 0.19)	0.10 (0.03. 0.14)*	0.13 (0.03. 0.29)*

Multiple child pregnancy					
No	871 (98.1%)	597 (96.8%)	307 (97.5%)	1671 (97.5%)	150 (96.8%)
Yes	17 (1.9%)	20 (3.2%)	8 (2.5%)	43 (2.5%)	5 (3.2%)
GH					
No	784 (91.0 %)	534 (88.7 %)	269 (88.8 %)	1488 (89.4%)	140 (92.7%)
Yes	78 (9.0 %)	68 (11.3 %)	34 (11.2 %)	176 (10.6%)	11 (7.3%)

Numbers did not equal 1909 women because of missing data on dental amalgam status or covariates.
GH = gestational hypertension with or without preeclampsia. BMI = Body mass index. SD = standard deviation. IQR = interquartile range
[a] p-values for the association with the dental amalgam status reported at first trimester visit: T-test or Mann-Whitney, ANOVA or Kruskal-Wallis for continuous variables, Chi-square test for categorical variables
[b] Values are n (%), unless otherwise started
[*] $p < 0.05$

Table 2. Mercury concentrations (µg/l) according to dental amalgam status.

Dental amalgam status	Mercury at 1st trimester visit		n*	p	Mercury at 3rd trimester visit		n*	p
	Median (IQR)	GM			Median (IQR)	GM		
Number reported at 1st trimester visit[a]				<0.001				<0.001
0	0.58 (0.24, 1.24)	0.51	886		0.46 (0.19, 0.90)	0.40	791	
1-4	0.74 (0.34, 1.42)	0.64	617		0.56 (0.29, 0.98)	0.51	552	
> 5	0.90 (0.56, 1.42)	0.86	317		0.71 (0.40, 1.24)	0.71	278	
Number reported at 3rd trimester visit[a]				<0.001				<0.001
0	0.60 (0.26, 1.26)	0.53	822		0.46 (0.19, 0.95)	0.41	820	
1-4	0.74 (0.34, 1.40)	0.64	548		0.54 (0.28, 0.96)	0.49	530	
> 5	0.90 (0.58, 1.49)	0.90	294		0.74 (0.40, 1.24)	0.71	286	
Replacement within 12 months prior to 1st trimester visit[b]				0.003				<0.001
No	0.68 (0.30, 1.36)	0.59	1713		0.54 (0.26, 0.98)	0.47	1526	
Yes	0.88 (0.42, 1.39)	0.81	155		0.64 (0.37, 1.33)	0.69	140	
Replacement during pregnancy reported at 3rd trimester visit[b]				0.006				0.002
No	0.68 (0.30, 1.36)	0.59	1696		0.56 (0.26, 0.98)	0.47	1509	
Yes	0.86 (0.40, 1.36)	0.79	173		0.59 (0.36, 1.18)	0.66	158	

* Numbers did not equal 1909 women because of missing data on dental amalgam status or mercury concentrations.
IQR = interquartile range. GM= geometric mean.
[a] Kruskal-Wallis test for the association between mercury and presence of dental amalgam.
[b] Mann-Whitney test for the association between mercury and dental amalgam replacement.

Table 3. Crude and adjusted Odds Ratios for the association between dental amalgam status and GH.

Dental amalgam status	GH					
	n	Unadjusted OR (95%CI)	p	n	Adjusted OR (95%CI)	p
Number reported at 1st trimester visit[a]	1769			1766		
0		Reference			Reference	
1-4		1.28 (0.91, 1.80)	0.16		1.31 (0.92, 1.85)	0.13
> 5		1.27 (0.83, 1.95)	0.27		1.32 (0.86, 2.04)	0.21
Number reported at 3rd trimester visit[b]	1634			1516		
0		Reference			Reference	
1-4		1.27 (0.89, 1.82)	0.19		1.26 (0.85, 1.88)	0.25
> 5		1.18 (0.75, 1.85)	0.47		1.03 (0.63; 1.70)	0.90
Replacement within 12 months prior to 1st trimester visit[c]	1815	0.66 (0.35, 1.25)	0.21	1724	0.75 (0.40, 1.42)	0.38
Replacement during pregnancy reported at 3rd trimester visit[d]	1816	0.65 (0.36, 1.20)	0.17	1725	0.73 (0.39, 1.34)	0.31

Numbers did not equal 1909 women because of missing data on dental amalgam status, GH or covariates on multivariate models.
GH = gestational hypertension with or without preeclampsia. OR = odds ratio. CI = confident interval.
Logistic regression between dental amalgam status and GH:
[a] adjusted for maternal age
[b] adjusted for maternal age, BMI
[c,d] adjusted for maternal age, fish consumption

Table 4. Association between concurrent measures of dental amalgam status and GH (including 1st and 3rd trimester visits) among 1909 pregnant women.

Dental amalgam status	GH			
	Unadjusted OR (95%CI)	p	Adjusted OR (95%CI)	p
Number of dental amalgam[a]	1.03 (1.00, 1.05)	0.05	1.02 (0.99, 1.05)	0.17
Replacement before or during pregnancy[b]	0.99 (0.99, 1.00)	0.13	0.99 (0.99, 1.00)	0.16

GH = gestational hypertension with or without preeclampsia. OR = odds ratio. CI = confident interval.
Logistic generalized estimating equations between dental amalgam status and GH:
[a] adjusted for maternal age, BMI, education, household income, ethnicity, fish consumption, weight gain, coffee intake
[b] adjusted for maternal age

Chapitre 7 - Association between maternal exposures to bisphenol A or triclosan and gestational hypertension and preeclampsia: The MIREC Study (Article 3)

Short title: Bisphenol A and triclosan and hypertensive disorders of pregnancy

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Contribution des auteurs :

- Louopou Rosalie Camara a constitué la base de données, elle a fait toutes les analyses statistiques ainsi que la rédaction du manuscrit sous la supervision de Dre Helen Trottier et de Dr William D. Fraser.

- Les coauteurs : Dre Helen Trottier a participé aux analyses et à la rédaction du manuscrit. Dr William D. Fraser est l'un des principaux chercheurs de l'étude MIREC. Ils ont participé à la conception et au design de l'étude, à sa conduite, à la rédaction du manuscrit et aux interprétations des données.

Abstract

Background: Little is known about the association between bisphenol A (BPA) or triclosan (TCS) exposure and hypertension in pregnancy.

Objective: To investigate potential associations between maternal urinary concentrations of BPA or TCS and gestational hypertension and preeclampsia in a Canadian cohort of pregnant women.

Methods: Among 1909 pregnant women participating in MIREC Study, urinary concentrations of BPA and TCS were measured in the first trimester by liquid chromatography-tandem mass spectrometry using isotope dilution. Blood pressure was measured during each trimester. Multinomial regression was performed to estimate the adjusted odds ratio (aOR) and 95% confidence intervals (CI) for the associations between these phenols and gestational hypertension and preeclampsia.

Results: BPA urinary concentrations were similar across different categories of hypertension status: the median (interquartile range) concentrations were 0.97 µg/l (0.40, 2.00), 1.10 µg/l (0.40, 1.90), and 0.80 µg/l (0.35, 1.70) for women with gestational hypertension, those with preeclampsia and normotensive women, respectively (p = 0.075). For TCS, the values were 10.04 µg/l (2.56, 76.26) for the group with gestational hypertension, 6.48 µg/l (1.48, 67.94) for those with preeclampsia and 8.56 µg/l (2.16, 67.35) for normotensive women (p = 0.559). Compared with the lowest tertile, BPA and TCS were not associated with gestational hypertension (aOR for BPA > 1.30 µg/l: 1.00; 95% CI: 0.54-1.81 and for TCS > 32.6 µg/l: 1.41; 95% CI: 0.83-2.37) or preeclampsia (aOR for highest tertiles were 1.26; 95% CI: 0.53-2.97 and 0.68; 95% CI: 0.31-1.48, respectively).

Conclusion: BPA and TCS urinary concentrations measured during the first trimester of pregnancy were not associated with gestational hypertension or preeclampsia. Additional studies are required to confirm our results.

Introduction

Hypertension in pregnant women contributes to maternal morbidity (Lykke et al. 2009; Nakimuli et al. 2016; Riaz et al. 2011) and mortality (Berhan and Endeshaw 2015; Lo et al. 2013; Moodley 2004; Nakimuli et al. 2016; Riaz et al. 2011) and can occur in 10% of pregnancies (Duley 2009). Gestational hypertension and preeclampsia are included in this condition (Magee et al. 2014). Two to 8% of pregnancies are complicated by preeclampsia (Duley 2009). Preeclampsia and gestational hypertension rates have increased significantly (by 25 and 184%, respectively) over last 17 years (1987-2004) in the United States (Wallis et al. 2008). In Canada, the rate of gestational hypertension without proteinuria was 46.2 per 1000 deliveries in 2010-2011 (Public Health Agency of Canada, 2014). Several physiopathology mechanisms, including those associated with genetic, immune and vasoactive factors have been invoked (Ali and Khalil 2015; Powe et al. 2011; Robillard et al. 2011) but the etiology of hypertension in pregnancy still remains uncertain (Robillard et al. 2009; Robillard et al. 2011). Environmental factors have been associated with this outcome (Cantonwine et al. 2016; Cunningham et al. 2010; Kobashi 2006). Among possible environmental factors, exposure to chemicals such as bisphenol A (BPA) and triclosan (TCS) are of particular interest for their widespread use in consumer products (FDA 2016a; FDA 2016b; GOC 2016; GOC 2017).

BPA is an industrial chemical used to make polycarbonate plastics (GOC 2017). It is biologically active (Gassman et al. 2015). The absence of enterohepatic circulation in humans can cause rapid conjugation and excretion of BPA (Völkel et al. 2002). The glucuronide form of BPA is the predominant form found in urine (Provencher et al. 2014). Exposure to BPA commonly occurs through the oral route (dietary exposure) (Kang et al. 2006; Vandenberg et al. 2007; von

Goetz et al. 2017) followed by the skin (dermal exposure) (von Goetz et al. 2017). Both contribute around 90% and 10% respectively to internal exposure to total (conjugated plus unconjugated) BPA (von Goetz et al. 2017). It is the most common monomer among polycarbonates intended for food contact (Health Canada 2014). BPA is also present in a wide range of consumer products (FDA 2016b; GOC 2017; Health Canada 2012).

Although not consistent in the literature (Bae and Hong 2015; Cantonwine et al. 2016; Shiue 2014a; Wang et al. 2015), BPA has been associated with human conditions such as preeclampsia (Leclerc et al. 2014), hypertension (Bae and Hong 2015), cardiovascular diseases, obesity, diabetes type 2, respiratory diseases, chronic kidney diseases, birth defects and development disorders, behavioral disorders, cancer and autoimmune diseases (Rezg et al. 2014). To date, only two studies (Cantonwine et al. 2016; Leclerc et al. 2014) have examined the association between BPA and hypertension in pregnant women and both have reported an increased risk for preeclampsia, dependent on the matrix in which BPA was measured.

Humans are also exposed to TCS, the use of which has become increasingly widespread since 1972 (Bergstrom 2014). TCS is an antimicrobial agent used in health and personal hygiene products (Bergstrom 2014; FDA 2016a; GOC 2016), clothing, kitchenware, furniture and toys (FDA 2016a). It has no known natural sources and its presence in the environment is exclusively due to anthropogenic activity (ECCC 2017).

TCS is quickly absorbed from the gastrointestinal tract and conjugated by the hepatic uridine diphosphate-glucuronyltransferases into its glucuronidated forms (Sandborgh-Englund et al. 2006). Glucuronide metabolites are the most predominant species of TCS in urine samples (Provencher et al. 2014). TCS has high hydrophobicity and as a consequence (Bedoux et al.

2012), it has been found in human plasma (Allmyr et al. 2006), urine (Lu et al. 2016; Provencher et al. 2014), and breast milk (Adolfsson-Erici et al. 2002; Allmyr et al. 2006).

TCS toothpaste has been associated with lower cardiovascular disease biomarkers (total, high density lipoprotein and low density lipoprotein cholesterol) (Cullinan et al. 2015). A few studies have analyzed the association between TCS and hypertension in the general population (Shiue 2014b; Shiue and Hristova 2014) without finding a significant relationship and no study to date has examined this association among pregnant women.

Our objective was to analyze the potential association between maternal urinary concentrations of BPA or TCS and the development of gestational hypertension or preeclampsia in a prospective pregnancy cohort.

Methods

Study design and population

The MIREC (Maternal-Infant Research on Environmental Chemicals) Study is a prospective cohort of 2001 pregnant women recruited in 10 Canadian cities (Vancouver, Edmonton, Winnipeg, Sudbury, Ottawa, Kingston, Hamilton, Toronto, Montréal, and Halifax). Women with no serious medical conditions, aged ≥ 18 years, and able to communicate in French or English were recruited in the first trimester of pregnancy. Women who withdrew from the study (n = 18) or suffered a miscarriage or stillbirth (n = 74) were excluded from our analysis, leaving a sample size of 1909 participants. Full details of the MIREC Study population have been published by Arbuckle et al. (2013). Maternal urine was collected during the first trimester visit (recruitment) and analysed for BPA and TCS. Blood pressure was measured during each trimester of pregnancy as well as after hospital admission for delivery. The women completed questionnaires on

sociodemographic and exposure characteristics and clinical data throughout pregnancy were abstracted from the medical charts. The study was approved by Health Canada's Research Ethics Board and the Research Ethics Committee of Sainte-Justine University Hospital in Montreal, Quebec (Canada) as well as in all MIREC affiliated recruitment centers. Consent forms were signed by all participants.

BPA and TCS measurements

One sample of urine was collected from each participant during the first trimester of pregnancy and analyzed for BPA (free, glucuronide, disulfate and monosulfate) and TCS (free, glucuronide and sulfate) by liquid chromatography coupled with tandem mass spectrometry (LC-MS/MS) (Provencher et al. 2014). Specific gravity was measured by refractometry (UG-1, Atago # 3461, Atago U.S.A. Inc., Bellevue WA, USA) to adjust for varying urinary dilution among the samples. All analyses were performed at the Centre de Toxicologie du Québec, Institut National de Santé Publique du Québec (INSPQ), Quebec, Canada.

Total BPA was the sum of free BPA, glucuronide, disulfate and monosulfate. Similarly total TCS was the sum of free, glucuronide and sulfate. Limits of detection (LOD) based on the metabolite with the highest LOD were: 0.47 µg/l for total BPA and 0.12 µg/l for total TCS. A value equivalent to the half the LOD was attributed to BPA and TCS concentrations below the LOD.

Blood pressure and Diagnosis of hypertension

Systolic and diastolic blood pressure (SBP and DBP) were assessed at each clinic visit by sphygmomanometer with the participant in a sitting position, with the cuffed arm resting on a desk at the level of the heart, by the clinical staff. Two measures of blood pressure were taken

about 1 minute apart and averaged for each visit. The Korotkoff phase V (disappearance) was used for DBP measurement. The diagnosis of hypertension was based on the guideline of the Society of Obstetricians and Gynaecologists of Canada guidelines (Magee et al. 2014), that is the average of two measurements of SBP equal to or greater than 140 mmHg and /or DBP equal to or greater than 90 mmHg, at gestational age equal to or greater than 20 weeks. The presence of proteinuria or maternal complications defines preeclampsia whereas their absence defines gestational hypertension without preeclampsia (Magee et al. 2014). Gestational age measured in weeks was based on last menstrual period, corrected by early ultrasound result, as required. In our study, 129 (6.8%) women were diagnosed with gestational hypertension without preeclampsia and 58 (3%) were diagnosed as having preeclampsia.

Covariates

Maternal age at delivery in years (18–31, 32–35, 36-49), parity (multiparous, nulliparous), ethnicity (caucasian, non-caucasian), pre-pregnancy body mass index (underweight, normal weight, overweight, obese), weight gain during pregnancy (difference between last weight measured prior to delivery and weight measured at first trimester visit considered in categorical form: <12.40, 12.40-17.24, >17.24 kg), education (university, college, less than college), household income in Canadian dollars ($ < 65,000, 65,000 – 90,000, > 90,000), maternal smoking (never, quit before pregnancy, quit during pregnancy, currently smoking) were considered as potential covariates and extracted from administrated questionnaires and medical chart reviews. Specific gravity was used as covariate in the models to account for the effect of urinary dilution.

Statistical analysis

Descriptive statistics were performed to analyze participant characteristics according to BPA and TCS urinary levels using Mann-Whitney and Kruskal-Wallis tests. Median (interquartile range (IQR)) and categorical distribution of BPA and TCS were determined according to the hypertension status (normotensive, gestational hypertension or preeclampsia). Median (IQR) and tertile distributions were compared using Kruskal-Wallis and chi-square tests, respectively. The Spearman correlation was calculated to examine the relationship between urinary BPA and TCS concentrations.

Multinomial regression models were performed to compute odds ratios (OR) and 95% confidence intervals (CI) for the association between BPA and TCS and hypertension status. BPA and TCS were individually tested in each model as a continuous and as a categorical variable (tertiles) in order to consider different alternatives for modeling exposure. Maternal age, maternal smoking, household income and education were identified in the MIREC study by Arbuckle et al. (2015) as predictors for BPA and TCS and were considered a priori as potential confounding variables and included in multivariate models because they are also implicated in hypertensive disorders based on published work. In addition, all other above-mentioned covariates were examined empirically as potential confounders using Change-in-estimates Method. Those that changed by +/-10% the OR for the association between BPA or TCS and hypertension status were considered as a confounder and included in each corresponding multivariate model. In order to optimize power multivariate models, covariates were considered in a continuous form when possible and missing data were ignored. This may have reduced the number of women in certain multivariate models but as missing data were rare and considered "missing completely at random (MCAR)", it did not have an important impact on results. A p-

value <0.05 was considered to indicate statistical significance. All statistical analyses were performed with IBM SPSS Statistics Version 22, SAS 9.4 for Windows and R for Windows 3.2.2.

Results

Table 1 shows the urinary concentrations of BPA and TCS according to participant characteristics. BPA concentrations were significantly higher in pregnant women aged between 18-31 years (median 0.95 µg/l) compared to those between 32-35 years (0.81 µg/l) and 36-49 years (0.71 µg/l). Obese pregnant women had the highest concentration of BPA (median 1.20 µg/l) compared to underweight, normal weight or overweight pregnant women (0.77 µg/l, 0.71 µg/l, and 0.86 µg/l, respectively). BPA concentrations were also significantly higher in women with lower household income.

Urinary TCS concentrations were significantly higher in non-caucasian (median 14.02 µg/l) compared to Caucasian women (8.26 µg/l), as well as among women with the highest household income and those who had never smoked. BPA and TCS concentrations were significantly but weakly correlated ($r = 0.20$, $p < 0.001$).

Table 2 presents concentrations of BPA and TCS according to hypertension status (normotensive, gestational hypertension without preeclampsia and preeclampsia). The median concentrations of BPA or TCS did not differ significantly by hypertension status. Similarly, when hypertension status was examined by tertiles of BPA or TCS, no statistically significant differences were observed (Table S1).

Tables S2 and 3 show the unadjusted and adjusted odds ratios (OR) for the association between BPA or TCS and hypertension status. Neither BPA nor TCS were significantly

associated with gestational hypertension or preeclampsia when examined as tertiles or as a continuous variable.

Discussion

BPA

To our knowledge, only two previous studies have examined the association between BPA and preeclampsia among pregnant women. Our results were consistent with those reported in a case-control study by Leclerc et al. (2014) who reported that BPA concentrations (median (min-max)) measured in the serum of 23 preeclamptic women (2.80 µg/l (0.21-9.00)) were similar to those of 35 normotensive women (3.00 µg/l (0.04-24.2)). Similar results were reported for BPA concentrations in fetal serum; however, a significantly higher concentration of BPA was measured in the placenta of preeclamptic women (9.40 µg/l (0.40–101)) compared to the placenta of normotensive women (3.00 µg/l (0.30-36.1)).

In the nested case-control study of Cantonwine et al. (2016) including 482 women with 50 cases of preeclampsia, an increase in urinary concentration of BPA was associated with an increased risk of preeclampsia (adjusted hazard ratio for an interquartile range increase of urinary concentrations of BPA = 1.53; 95% CI: 1.04-2.25). The specific-gravity adjusted geometric mean BPA concentration in our MIREC study (0.90 µg/l) was lower than that in the Cantonwine et al. (2016) study (1.34 µg/l) which may at least partially explain the discordant results between studies.

Studies of the association between phenols and blood pressure among the general population are inconsistent and were mostly cross-sectional in nature. For example, no association between urinary BPA and high blood pressure (OR = 1.12; 95% CI: 0.93-1.35) was

found in the 2009-2010 US National Health and Nutritional Examination Survey (NHANES) (Shiue 2014a) or in the 2009-2012 US NHANES (OR = 1.13; 95% CI: 0.98-1.31) (Shiue and Hristova 2014). In the Korean Elderly Environmental Panel Study (2008-2010) with 521 subjects ≥ 60 years old and a mean urinary concentration of BPA of 1.2 μg/g creatinine, Bae et al. (2012) reported no association between the fourth quartile of urinary BPA concentration (≥ 1.33 μg/g of creatinine) and hypertension (OR = 1.27; 95% CI: 0.85-1.88); however, when restricted to participants without previous history of hypertension (n = 258), an association was observed. Using data from the Thai National Health Examination Survey IV, 2009 (n = 2588), Aekplakorn et al. (2015) reported no significant association between serum BPA and hypertension in premenopausal women (OR = 2.12; 95% CI: 0.87-5.19) in contrast to postmenopausal women and in men (OR = 1.44; 95% CI: 0.99-2.09) compared to women. On the other hand, some studies have observed that BPA was associated with an increased risk of hypertension in the general population. Bae and Hong (2015) conducted a randomized crossover trial (n = 60 and ≥ 60 years old) and found an increase of 4.51 mmHg in SBP after drinking 2 canned beverages (mean = 16.91 ± 12.55 μg/l in urinary BPA concentration) compared with drinking 2 glasses of bottled beverages (mean = 1.13 ± 1.76 μg/l). Shankar and Teppala (2012) found an association between the third tertile urinary BPA (> 4.0 μg/l) and hypertension in the US NHANES 2003-2004 (n = 1380). Conversely, a cross-sectional study (n = 3246 and ≥ 40 years old) observed a decreased risk of hypertension between the highest tertile of urinary BPA (> 1.45 μg/l) and hypertension (OR = 0.61; 95% CI: 0.46-0.80) (Wang et al. 2015).

Unlike our cohort study, the majority of the studies in the general population, cited above, were conducted in participants aged ≥ 40 years old with a cross-sectional study design. Cross-

sectional studies can only provide a 'snapshot' of the exposure-outcome at a given time point and provide no indication of the sequence of events unless it is serial (Levin 2006) contrary to cohort studies (Song and Chung 2010). The Bae et al. (2012) and Bae and Hong (2015) studies were conducted in older populations ≥ 60 years with higher urinary concentrations of BPA. This older population is more susceptible to develop cardiovascular disease as age is a cardiovascular risk factor (Dhingra and Vasan 2012). Also mean urinary concentration of BPA was higher in Bae et al. (2012) study (1.2 µg/g creatinine) compared to MIREC study (specific-gravity corrected geometric mean = 0.90 µg/l), The Aekplakorn et al. (2015) study measured serum BPA and there is concern about the validity of serum measures of BPA because of the potential for contamination (Calafat et al. 2013).

Despite the fact that we found no association between BPA and gestational hypertension or preeclampsia, previous studies have reported that the toxicity of BPA might occur through a mechanism of oxidative stress (Asimakopoulos et al. 2015; Rezg et al. 2014) which may appear through an increase of urinary markers of oxidative stress (8-hydroxydeoxyguanosine and isoprostane) (Watkins et al. 2015), reactive oxygen species and a reduction of glutathione (Xin et al. 2014). Oxidative stress can increase circulating levels of the anti-angiogenic factor (fms-like tyrosine kinase-1) (Cindrova-Davies 2009), lead to endothelial dysfunction (Cindrova-Davies 2009; Myatt and Cui 2004; Reslan and Khalil 2010) and systemic vasoconstriction (Khalil and Granger 2002). These mechanisms are associated with hypertension in pregnancy or preeclampsia (Ali and Khalil 2015; Cindrova-Davies 2009).

BPA might also increase the mRNA expression of the proangiogenic genes (endothelial nitric oxide synthase, vascular endothelial growth factor A, and vascular endothelial growth

receptor 2) and the production of nitric oxide in human umbilical vein endothelial cells (Andersson and Brittebo 2012). It may target human endothelium. Endothelial dysfunction may play a key role on the effect of BPA on cardiovascular system (Andersson and Brittebo 2012). However, others studies in rodents (rats or murine) have suggested that BPA might decrease a nitric oxide production (Aboul Ezz et al. 2015; Byun et al. 2005), inhibit acetylcholinesterase activity (Aboul Ezz et al. 2015) and suppress tumor necrosis factor-alpha synthesis (Byun et al. 2005). Some of these factors (endothelial dysfunction and low nitric oxide) are associated with hypertension in pregnancy or preeclampsia (Ali and Khalil 2015). The discrepancy between this potential for biological plausibility and our results may be explained by: 1) the lower concentration of BPA in our study may not be sufficient to pose a health risk; 2) the animal models may not be pertinent to humans; or 3) differences in the study populations such as genetic predisposition leading to variability in the response to BPA exposure. It is not expected that methodological differences between Canada and the US would significantly impact the higher urinary BPA concentrations reported in the US than in Canada (Lakind et al. 2012), which might suggest that there are population differences (e.g., consumer product formulations or avoidance of BPA-containing products) in Canada (Arbuckle et al. 2015).

TCS

No association between TCS and hypertension status among pregnant women was found in our study, nor to our knowledge, has any other study examined such an association. Two cross-sectional analyses (Shiue 2014b; Shiue and Hristova 2014) also reported no association between TCS and high blood pressure.

Strengths and limitations of the study

To our knowledge this is the first study examining the association between TCS and hypertension in pregnant women. Strengths of the study include the relatively large sample size (n = 1909), its prospective cohort design and recruitment from multiple sites across Canada. The potential confounding variables were conservatively controlled. All laboratory analysis were performed by the Centre de Toxicologie du Québec which is a national reference laboratory. However there are some limitations related to BPA and TCS measurements in urine. Both phenols were measured only once during the first trimester while BPA and TCS have a short half-life (<2 h for BPA (WHO 2010) and 10.8h for TCS (Queckenberg et al. 2010)). This can underestimate exposure among women in the study.TCS has a temporal variability as illustrated by the intraclass correlation coefficients (ICC) ranging from high (within a week-day (0.77) and week-end day (0.79)) to moderate across the study period (0.50) (Weiss et al. 2015) or for three time points during pregnancy (0.49) (Bertelsen et al. 2014). In contrast to TCS, Fisher et al. (2015) found reduced reproducibility for BPA (within a weekday (ICC = 0.33), a weekend day (ICC = 0.31) and across pregnancy (ICC = 0.07)), but a 65% sensitivity to correctly classify a participant in the 'high' category using a randomly chosen sample versus the GM of all the urine samples. Another limitation of this study is that the majority of the participants was Caucasian, university educated, non-smokers and had high household income; therefore, the generalizability of our results may be limited.

Conclusion

Our findings suggest no significant association between BPA or TCS concentrations in pregnant women and the risk of gestational hypertension or preeclampsia. Further studies are needed to confirm our results.

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Table 1. Participant characteristics according to urinary BPA and TCS concentrations.

Characteristics	n*	BPA (µg/l) Median (IQR)	p[a]	n*	TCS (µg/l) Median (IQR)	p[a]
Maternal age (years)			<0.001			0.797
18 - 31	737	0.95 (0.40, 1.90)		713	8.57 (2.29, 60.17)	
32 - 35	528	0.81 (0.36, 1.60)		513	9.43 (2.12, 80.22)	
36 - 49	590	0.71 (0.33, 1.50)		564	8.30 (2.16, 71.81)	
Weight gain (kg)			0.103			0.409
<12.40	564	0.90 (0.39, 1.90)		547	9.74 (2.35, 66.27)	
12.40-17.24	562	0.75 (0.35, 1.60)		541	8.88 (2.25, 73.33)	
>17.24	563	0.82 (0.35, 1.60)		543	7.28 (1.98, 65.57)	
Pre-Pregnancy body mass index			<0.001			0.051
Underweight	51	0.77 (0.25, 1.55)		45	8.02 (3.31, 149,55)	
Normal weight	1049	0.71 (0.33, 1.50)		1011	7.91 (1.99, 56.42)	
Overweight	373	0.86 (0.40, 1.90)		365	7.94 (2.32, 66.94)	
Obese	254	1.20 (0.43, 2.20)		247	13.64 (2.94, 82.34)	
Ethnicity			0.792			0.009
Caucasian	1603	0.83 (0.36, 1.70)		1554	8.26 (2.11, 62.86)	
Non-caucasian	261	0.81 (0.39, 1.70)		245	14.02 (2.81, 140.52)	
Education			<0.001			0.070
University	1165	0.73 (0.33, 1.60)		1131	9.44 (2.32, 84.32)	
College	496	0.89 (0.37, 1.80)		475	7.88 (1.92, 51.28)	
Less than college	201	1.30 (0.68, 2.50)		191	6.54 (2.01, 46.17)	
Household income ($CAN)			<0.001			0.035
< 65,000	537	1.20 (0.46, 2.10)		513	7.90 (2.07, 55.22)	
65,000 – 90,000	518	0.84 (0.32, 1.70)		505	7.67 (2.01, 51.91)	
> 90,000	722	0.67 (0.32, 1.40)		702	10.61 (2.45, 98.62)	
Parity			0.961			0.417
Multiparous	1050	0.82 (0.35, 1.70)		1006	8.53 (2.16, 65.93)	
Nulliparous	812	0.82 (0.37, 1.70)		791	8.97 (2.30, 83.03)	

Maternal smoking			0.132			0.010
Never	1139	0.78 (0.34, 1.60)		1094	10.26 (2.44, 83.57)	
Quit before Pregnancy	607	0.86 (0.38, 1.80)		594	7.42 (1.96, 53.18)	
Quit during Pregnancy	20	0.76 (0.35, 1.60)		20	3.99 (1.75, 281.23)	
Currently Smoking	97	1.20 (0.58, 2.10)		90	5.25 (1.96, 44.89)	

* Numbers did not equal 1909 women because of missing data on phenols or covariates
BPA=bisphenol A. TCS=triclosan. IQR= interquartile range.
[a] p-values for kruskal-wallis and Mann-Whitney tests for the association with BPA or TCS.

Table 2. Concentrations of urinary BPA and TCS by hypertensive status.

	Normotensive		Gestational hypertension		Preeclampsia		p[a]
	n*	Median (IQR)	n*	Median (IQR)	n*	Median (IQR)	
BPA (μg/l)	1591	0.80 (0.35, 1.70)	128	0.97 (0.40, 2.00)	57	1.10 (0.40, 1.90)	0.075
TCS (μg/l)	1534	8.56 (2.16, 67.35)	128	10.04 (2.56, 76.26)	55	6.48 (1.48, 67.94)	0.559

* Numbers did not equal 1909 women because of missing data on phenols or gestational hypertension and preeclampsia. BPA=Bisphenol A. TCS=triclosan. IQR= interquartile range.

[a] p-values for Kruskal-Wallis test between BPA or TCS and normotensive, gestational hypertension and preeclampsia.

Table 3. Adjusted OR (95%) for the association between BPA or TCS and gestational hypertension and preeclampsia.

Urinary Concentration (µg/l)	n	**Gestational hypertension** **Adjusted OR (95% CI)[a]**	p	**Preeclampsia** **Adjusted OR (95% CI)[a]**	p
BPA (continuous form)	1541	0.98 (0.92, 1.03)	0.403	1.00 (0.95, 1.05)	0.864
BPA	1541				
< 0.50		Reference		Reference	
0.50 – 1.30		0.94 (0.55, 1.61)	0.819	0.99 (0.44, 2.24)	0.977
> 1.30		1.00 (0.54, 1.81)	0.999	1.26 (0.53, 2.97)	0.602
TCS (continuous form)	1496	1.00 (1.00, 1.00)	0.974	1.00 (1.00, 1.00)	0.547
TCS	1496				
< 3.60		Reference		Reference	
3.60 – 32.60		1.38 (0.82, 2.31)	0.223	0.73 (0.35, 1.52)	0.397
> 32.60		1.41 (0.83, 2.37)	0.202	0.68 (0.31, 1.48)	0.325

Numbers did not equal 1909 women because of missing data on BPA, TCS, covariates or gestational hypertension and preeclampsia on the multivariate models. OR=odds ratio. CI= confident interval. BPA= bisphenol A. TCS=triclosan.

[a] Multinomial regression (between BPA or TCS and gestational hypertension and preeclampsia versus normotensive) adjusted for maternal age, smoking status, education, household income, pregnancy weight gain and specific gravity.

Table S1. Distribution of hypertension status by tertiles of urinary BPA and TCS.

	Normotensive n (%)	**Gestational hypertension n (%)**	**Preeclampsia n (%)**	**P[a]**
BPA (µg/l)				0.342
< 0.50	548 (34.4%)	40 (31.3%)	16 (28.1%)	
0.50 – 1.30	534 (33.6%)	39 (30.5%)	17 (29.8%)	
> 1.30	509 (32.0%)	49 (38.3%)	24 (42.1%)	
TCS (µg/l)				0.403
< 3.60	523 (34.1%)	35 (27.3%)	23 (41.8%)	
3.60 – 32.60	509 (33.2%)	47 (36.7%)	16 (29.1%)	
> 32.60	502 (32.7%)	46 (35.9%)	16 (29.1%)	

Numbers did not equal 1909 women because of missing data on phenols or gestational hypertension and preeclampsia.
BPA=bisphenol A. TCS=triclosan.
[a] Chi-square test between BPA or TCS and normotensive, gestational hypertension and preeclampsia.

Table S2**.** Crude OR (95%) for the association between BPA or TCS and gestational hypertension and preeclampsia.

Urinary concentration (µg/l)	n	**Gestational hypertension** **Unadjusted OR (95% CI)[a]**	p	**Preeclampsia** **Unadjusted OR (95% CI)[a]**	p
BPA (continuous form)	1776	0.99 (0.95, 1.03)	0.521	1.00 (0.96, 1.04)	0.911
BPA	1776				
< 0.50		Reference		Reference	
0.50 – 1.30		1.00 (0.63, 1.58)	0.998	1.09 (0.55, 2.18)	0.807
> 1.30		1.32 (0.85, 2.04)	0.212	1.62 (0.85, 3.06)	0.145
TCS (continuous form)	1717	1.00 (1.00, 1.00)	0.993	1.00 (1.00, 1.00)	0.669
TCS	1717				
< 3.60		Reference		Reference	
3.60 – 32.60		1.38 (0.88, 2.17)	0.165	0.72 (0.37, 1.37)	0.311
> 32.60		1.37 (0.87, 2.16)	0.177	0.73 (0.38, 1.39)	0.331

Numbers did not equal 1909 women because of missing data on BPA, TCS or gestational hypertension and preeclampsia.
OR=odds ratio. CI= confident interval. BPA= bisphenol A. TCS=triclosan.
[a]Multinomial regression between BPA or TCS and gestational hypertension and preeclampsia versus normotensive

Chapitre 8 - Discussion Générale

En complément aux discussions des trois articles, nous ferons dans cette section un survol des principaux résultats, de la validité, des forces de l'étude ainsi que des possibles implications pour la santé publique et les recherches futures.

8.1 Aperçu des principaux résultats

Les métaux et les désordres hypertensifs de la grossesse

Le Mn sanguin

Dans notre étude, nous avons observé que le Mn sanguin mesuré au premier trimestre (> 9.89 µg/l) était associé à un risque réduit de l'hypertension gestationnelle globale (OR ajusté (aOR) = 0.68; 95% IC : 0.46, 0.99) et de la prééclampsie (aOR = 0.48; 95% IC : 0.23, 0.98). Cependant, l'association avec le Mn était modifiée par le sexe de l'enfant. Le Mn (> 9.89 µg/l) était inversement lié au risque d'hypertension gestationnelle globale (aOR = 0.41; 95% IC : 0.24, 0.70) et de l'hypertension gestationnelle (aOR = 0.41; 95% IC : 0.22, 0.75) lorsque l'enfant à naître était de sexe masculin. L'effet du Mn a disparu quand l'enfant à naître était de sexe féminin. L'interaction n'était pas significative pour le Mn mesuré au troisième trimestre.

Quand nous avons effectué une analyse de sensibilité en incluant tous les métaux sanguins dans le même modèle selon le trimestre de mesure, le Mn du premier trimestre était associé à une diminution de risque pour l'hypertension gestationnelle globale (aOR = 0.93; 95% IC : 0.87, 0.99). Lorsque les mesures concurrentes des métaux et de la TAM étaient analysées, le Mn était associé à une augmentation de la TAM (beta = 1.52; 95% IC : 0.80, 2.24). Cette discrépance dans les résultats du Mn peut être possiblement due aux caractéristiques des mesures des deux issues

qui sont différentes (la TA et les désordres hypertensifs de la grossesse). Les désordres hypertensifs de la grossesse sont des issues qui ont été mesurées à partir de la 20^{ième} semaine de la grossesse avec des caractéristiques différentes (TA ≥140/90 mmHg et/ou protéinurie ou complications maternelles à partir des 20 semaines de grossesse) de celles de la TAM (TAS + 2 TAD)/3) qui a été mesurée dès les premières semaines de la grossesse. Les désordres hypertensifs de la grossesse constituent ainsi un diagnostic d'hypertension artérielle tandis qu'une élévation de la TAM n'a pas nécessairement de signification clinique. Il est possible que le Mn augmente la TAM sans pour autant conduire à un diagnostic d'hypertension artérielle. Il est important de spécifier que les associations mesurées pour la TAM incluent les femmes avec l'hypertension chronique (qui sont traitées comme des données manquantes dans la définition des désordres hypertensifs de la grossesse). Cela aurait pu expliquer la différence dans les résultats utilisant les mesures concurrentes de la TAM et des métaux. Cependant, cela ne semble pas expliquer la différence car l'analyse de sensibilité avec l'exclusion des femmes avec l'hypertension chronique a donné des résultats similaires. Par ailleurs, le Mn a une demi-vie de 4-39 jours pour la clairance du corps entier (Santé Canada 2016). Il est possible que les mesures concurrentes aient coincidé au temps de l'effet maximal du Mn tandis que celles des désordres hypertensifs de la grossesse sont apparues après ce temps.

Nos résultats étaient cohérents avec ceux d'une étude cas-témoins (N = 108) qui a rapporté que les concentrations sériques moyennes du Mn étaient significativement plus faibles chez les prééclamptiques (0.08 µg/l) par rapport aux contrôles (0.14 µg/l) (Sarwar et al. 2013). Des résultats similaires ont également été observés par Al-Jameil et al. (2014) et Maduray et al. (2017). Cependant, nos résultats contrastent avec ceux qui ont signalé une association entre le Mn

et le risque plus élevé des désordres hypertensifs de la grossesse. Dans une étude de cohorte de 364 femmes enceintes saines, Vigeh et al. (2013) ont observé que les concentrations sanguines moyennes du Mn maternel mesurées au premier (18.6 µg/l) et au deuxième trimestre (18.9 µg/l) étaient associées à un risque plus élevé d'hypertension gestationnelle. Les résultats du troisième trimestre n'ont pas été statistiquement significatifs. Des résultats similaires ont été rapportés par Vigeh et al. (2006). Ce contraste dans les résultats peut s'expliquer par les concentrations plus faibles de Mn dans notre étude (MG environ 8 µg/l) par rapport à celle de Vigeh et al. (2013). Il est possible que, à des niveaux trop élevés, le Mn puisse provoquer une hypertension gestationnelle. Dans la population générale, Lee et Kim (2011) ont trouvé une association entre le Mn et les niveaux élevés de la TAS et la TAD (MG était de 1.33 mg/dl chez les participants hypertendus). Bien que nos données suggèrent l'importance de niveaux adéquats de Mn pour réduire les risques d'hypertension gestationnelle et de prééclampsie, les données d'autres études suggèrent que si les niveaux sont trop élevés, cela peut augmenter le risque des désordres hypertensifs de la grossesse. Bien qu'on n'ait pas trouvé d'études ayant examiné l'effet modificateur potentiel du sexe du bébé sur l'association entre le Mn et les désordres hypertensifs de la grossesse, notre résultat était conforme à celui de Lee et al. (2015) qui ont trouvé un risque plus faible de TAS associé à l'exposition au Mn chez les hommes par rapport aux femmes. Cet effet modificateur du sexe du bébé peut être possiblement associé aux niveaux sériques de la gonadotropine chorionique humaine qui sont plus élevés chez les femmes ayant des fœtus femelles comparées à celles ayant des fœtus males (Adibi et al. 2015). La gonadotropine chorionique humaine peut augmenter l'ARNm de la superoxyde dismutase du Mn (MnSOD mRNA) (Sasaki et al. 1994), un antioxydant qui protège contre les dommages oxydatifs (Sugino

et al.1998) et conséquemment les désordres hypertensifs de la grossesse (Hansson et al. 2015). Des niveaux bas de la gonadotropine chorionique humaine ont été associés au risque de la prééclampsie précoce (Keikkala et al. 2013) tandis que des niveaux plus élevés étaient associés à une prééclampsie sévère (Zheng et al. 2016).

L'As sanguin et urinaire

L'As sanguin mesuré au troisième trimestre était associé à une augmentation du risque de la prééclampsie (aOR = 1.16; 95% IC : 1.03, 1.30). Quand nous avons inclus tous les métaux sanguins dans le même modèle selon le trimestre de mesure, l'As du premier trimestre était associé à une augmentation de risque pour la prééclampsie (aOR = 2.75; 95% IC : 1.13, 6.73) pour le troisième tertile (> 0.97 µg/l). Dans le modèle de stratification par le sexe du bébé, le deuxième tertile de l'As (0.61 - 0.97 µg/l) était associé à un risque élevé de l'hypertension gestationnelle globale (aOR = 1.85; 95%IC : 1.02, 3.36). Pour les mesures concurrentes des métaux et de la TA, l'As était associé à une diminution de la TA (beta = -0.59; 95% IC : -0.92, -0.26). Comme nous l'avons expliqué dans la section du Mn, la nature de la mesure des issues (TAM et désordres hypertensifs de la grossesse) peut être probablement à la base de cette différence dans les résultats. Aussi, la demi-vie de l'As inorganique est de 2-40 jours (Santé Canada 2006). Il est possible que les mesures concurrentes aient coincidé au temps de l'effet minimal de l'As et que celles des désordres hypertensifs de la grossesse soient apparues après ce temps.

À notre connaissance, il n'existe pas d'études de cohorte sur des femmes enceintes évaluant l'association entre l'As sanguin et les désordres hypertensifs de la grossesse. Cependant, nos résultats sont cohérents avec ceux d'une étude cas-témoins (n = 398 femmes non enceintes)

où les auteurs ont trouvé une association entre l'As mesuré dans les cheveux et un risque plus élevé d'hypertension (OR = 2.55; IC 95% 1.55, 4.20) (Yu et al. 2017), mais pas ceux d'une autre étude cas-témoins (Maduray et al. (2017) qui n'ont pas trouvé d'association entre l'As mesuré dans les cheveux et le sérum et la prééclampsie. Cette différence entre les resultats de notre étude et celle de Maduray et al. (2017) pourrait être due aux niveaux très faibles d'As dans le sérum des femmes prééclamptiques dans leur étude (0.06 ± 0.0 (0.06, 0.06) µg/l). La signification du deuxième tertile de l'As dans l'analyse de la stratification selon le sexe de l'enfant à naître, peut être dû au hasard (chance) car ni le troisième tertile ni la forme continue de l'As ne sont significatifs.

Quant aux mesures urinaires, l'As inorganique n'étaient pas associés au risque de désordres hypertensifs de la grossesse ou de la TAM. Le DMA (acide diméthylarsinique) urinaire n'était pas associé au risque de désordres hypertensifs de la grossesse mais il était lié à une diminution de la TAM (beta = -1.00; 95% IC : -1.52, -0.48). Une étude cas-témoins portant sur 306 femmes enceintes a révélé des concentrations similaires d'As urinaire chez les prééclamptiques (moyenne = 7.1 µg/l) et les femmes normotendues (moyenne = 6.78 µg/l) (Sandoval-Carrillo et al. 2016). De forme similaire, Jones et al. (2011) n'ont montré aucune association entre les concentrations totales d'As, l'As total moins l'arsénobétaïne ou le DMA et l'hypertension artérielle dans la population générale. Pour l'arsénobétaïne, nos résultats étaient aussi conformes à ceux d'autres études (Shiue 2014b, Shiue et Hristova 2014). Farzan et al. (2015) ont trouvé dans leur étude de cohorte sur les femmes enceintes (n = 514) une association entre l'As mesuré dans l'urine et les niveaux élevés de la TAS. En revanche, Shiue et Hristova (2014) ont trouvé une association entre les concentrations moyennes de DMA (5.72 µg/l) et la

TA. Des résultats similaires ont été trouvés par Shiue (2014a, 2014b). Cette disparité dans les résultats peut être due aux concentrations plus faibles de DMA dans notre étude (2.30 µg/l était la MG la plus élevée).

Le Hg sanguin

Le Hg sanguin mesuré au troisième trimestre était associé à une dimintion du risque de l'hypertension gestationnelle globale (aOR = 0.62; 95% IC : 0.39, 0.98) pour > 0.82 µg/l). L'analyse de sensibilité avec ajustement pour tous les métaux sanguins, le Hg du troisième trimestre s'était maintenu inversement associé à l'hypertension gestationnelle globale (aOR = 0.66; 95% IC : 0.47, 0.93). Le Hg n'était pas associé à la TA quand les mesures concurrentes des deux ont été analysées. Conformément à nos résultats, une étude transversale de 263 femmes enceintes a révélé que le Hg inorganique (MG = 0.13 µg/l) était associé à une baisse de la TAS (-1.18 mmHg (-3.72, 1.35)) et de la pression d'impulsion (-2.51 mmHg (-4.49, -0.53)) contrairement au méthylmercure (MG = 0.95 µg/l) qui était associé à une TAS plus élevée (Wells et al. 2017). Dans la population générale, Park et al. (2013) ont révélé que le Hg urinaire était associé à une diminution du risque d'hypertension (OR = 0.87; IC 95% 0.78, 0.99) alors que le Hg sanguin n'était pas associé au risque d'hypertension. D'autres auteurs ont rapporté des résultats mitigés (Goodrich et al. 2013; Mozaffarian et al. 2012; Nielsen et al. 2012).

Le Pb sanguin

Le Pb sanguin mesuré au premier ou au troisième trimestre n'était pas associé aux désordres hypertensifs de la grossesse ni à la TAM. Conformément à nos résultats, Maduray et al. (2017) ont trouvé des concentrations similaires de Pb sérique chez les prééclamptiques (0.20 ± 0.17 µg/l) et les femmes normotendues (0.16 ± 0.21 µg/l). Les niveaux de Pb sanguins sensiblement bas

dans notre étude (environ 0.58 µg/dl) par rapport à d'autres peuvent fournir une explication de la disparité des résultats. Par exemple, les niveaux moyens de Pb pour la prééclampsie étaient : 60.2 µg/dl (Ikechukwu et al. 2012), 37.68 µg/dl (Motawei et al. 2013) ou 1.2 µg/dl (Sowers et al. 2002). Pour l'hypertension gestationnelle, les niveaux moyens de Pb étaient : 9.6 µg/dl (Magri et al. 2003), 6.9 µg/dl (Rabinowitz et al. 1987), 5.7 µg/dl (Vigeh et al. 2004) ou MG = 1.9 µg/dl (Yazbeck et al. 2009). Certaines différences méthodologiques peuvent également expliquer l'écart entre notre étude et celle de Wells et al. (2011) qui ont trouvé une association entre le Pb mesuré dans le sang du cordon ombilical (MG = 0.66 µg/dl) et les élévations de la TA. Les mesures de la TA dans l'étude de Wells et al. (2011) ont été effectuées pendant le travail et l'accouchement, une période connue pour être stressante (Costa et al. 1988; Irestedt et al. 1982). En outre, une seule mesure de la TA et du Pb a été effectuée ce qui pourrait introduire une erreur de mesure et affecter potentiellement la validité de leurs résultats. En plus, ils ont utilisé une étude transversale qui ne donne qu'un portrait «instantané» de la relation de l'exposition et de l'issue à un moment donné et ne fournit aucune indication de la séquence des événements (Levin 2006) contrairement à l'étude de cohorte qui fait un suivi sur une période de temps d'un ensemble de personnes (Song and Chung 2010).

Le Cd sanguin

Le Cd sanguin mesuré au premier ou au troisième trimestre n'était pas associé aux désordres hypertensifs de la grossesse ni à la TAM. Les niveaux du Cd sanguin dans notre étude (MG = 0.2 µg/l) étaient inférieurs à ceux rapportés par d'autres études qui ont trouvé une association avec l'hypertension gestationnelle (moyenne = 1.9 µg/l (Kosanovic et Jokanovic 2007)) ou l'hypertension (moyenne = 1.67 µg/l (Eum et al. 2008)). En accord avec nos résultats, une étude

n'a révélé aucune association entre le Cd et l'hypertension gestationnelle (Yazbeck et al. 2009). Des résultats similaires ont été trouvés dans la population générale avec l'hypertension artérielle (Al-Saleh et al. 2006; Shiue et Hristova 2014).

Les amalgames dentaires et l'hypertension gestationnelle globale

Dans notre étude nous n'avons pas mis en évidence une association entre la présence des amalgames dentaires ou leur remplacement et l'hypertension gestationnelle globale. Pour les mesures du premier trimestre, les aOR étaient de 1.31; 95% IC : 0.92, 1.85 et de 1.32; 95% IC : 0.86, 2.04 pour les femmes ayant 1-4 et ≥ 5 amalgames dentaires, respectivement. L'aOR pour le remplacement d'amalgame était de 0.75; 95% IC : 0.40, 1.42. Pour les mesures du troisième trimestre, les aOR étaient de 1.26; 95% IC : 0.85, 1.88 et de 1.03; 95% IC : 0.63, 1.70 pour les femmes ayant 1-4 et ≥ 5 amalgames dentaires, respectivement ; tandis que l'aOR pour le remplacement était de 0.73; 95% IC : 0.39, 1.34. Ce résultat s'était maintenu même avec les mesures concurrentes de l'exposition et de l'issue (aOR = 1.02; 95% IC : 0.99, 1.05 pour la présence des amalgames dentaires et aOR = 0.99; 95% IC : 0.99, 1.00 pour le remplacement). Par ailleurs, comme le groupe hypertension gestationnelle globale englobe les femmes avec l'hypertension gestationnelle ou la prééclampsie, une analyse de sensibilité a été utilisée pour regarder les associations en séparant les cas d'hypertension gestationnelle de ceux de la prééclampsie et les résultats n'ont pas changé en montrant toujours une absence d'association.

À notre connaissance, aucune autre étude n'a analysé l'association entre l'amalgame dentaire et la TA chez les femmes enceintes. Dans la population générale, les études sont très rares. Seulement une étude a trouvé un lien entre les amalgames dentaires et l'augmentation de la TA (Siblerud 1990). Par contre, d'autres études ne concernant pas directement la TA, n'ont pas

trouvé d'association entre les amalgames dentaires et les maladies cardiovasculaires telles que l'infarctus du myocarde (Ahlqwist et al. 1993; Bengtsson et al. 2001).

Dans notre étude, les niveaux du Hg à la visite du troisième trimestre étaient inférieurs à ceux de la visite du premier trimestre selon le statut des amalgames dentaires. Cela suppose que le Hg passe au fœtus comme le montre certaines études qui ont rencontré des concentrations élevées du Hg dans le sang du cordon ombilical par rapport à celles de la mère (Arbuckle et al. 2016; Chen et al. 2014; Sakamoto et a. 2012; Santos et al. 2007). La corrélation entre le Hg et les amalgames dentaires était relativement faible dans notre étude (Spearman's rho (r) = 0.156 comme valeur maximale) contrairement à d'autres études qui ont trouvé des corrélations fortes (Fakour et al. 2010a, 2010b) et malgré cette corrélation, aucune association n'a été montré dans notre étude entre les amalgames (présence ou remplacement) et l'hypertension gestionnelle globale.

Des études ont été menées sur l'association entre le Hg et la TA dans la population générale, chez les femmes enceintes et les enfants. La relation entre le Hg et la TA n'est pas consistente dans la littérature car certaines études ont observé des associations négatives (Park et al. 2013; Nielsen et al. 2012; Wells et al. 2017) et d'autres, des associations positives (Pan et al. 2007; Valera et al. 2008) ou une absence d'association (Gregory et al. 2016; Johansson et al. 2002; Kalish et al. 2014; Miller et al. 2017; Valera et al. 2011; Valera et al. 2012; Wells et al. 2017). Tels que discutés dans la section du Hg, nos résultats ont montré une association négative entre le Hg et les désordres hypertensifs de la grossese. L'absence d'association entre les amalgames dentaires et l'hypertension gestationnelle globale pourrait s'expliquer par la faible corrélation entre le Hg et les amalgames dentaires dans notre étude.

Le BPA ou le TCS et l'hypertension gestationnelle et la prééclampsie

Le BPA

Les niveaux du BPA n'étaient pas associés au risque de l'hypertension gestationnelle (aOR pour le troisième tertile (> 1.30 µg/l) = 1.00 95% IC : 0.54, 1.81) ou de la prééclampsie (aOR pour le troisième tertile = 1.26; 95% IC : 0.53, 2.97). Nos résultats étaient cohérents avec ceux rapportés dans une étude cas-témoins par Leclerc et al. (2014) qui ont signalé que les concentrations de BPA (médiane (min-max)) mesurées dans le sérum de 23 femmes prééclamptiques (2.80 µg/l (0.21-9.00)) étaient similaires à celles de 35 femmes normotendues (3.00 µg/l (0.04 -24.2)). Des résultats similaires ont été rapportés pour les concentrations de BPA dans le sérum fœtal ; cependant, une concentration significativement plus élevée de BPA a été mesurée dans le placenta chez les femmes prééclamptiques (9.40 µg/l (0.40-101) par rapport au placenta de femmes normotensives (3.00 µg/l (0.30-36.1)). Dans l'étude de cas-témoins nichée de Cantonwine et al. (2016), dont 482 femmes avec 50 cas de prééclampsie, une augmentation de la concentration urinaire de BPA était associée à un risque accru de prééclampsie pour chaque augmentation de la concentration médiane urinaire de BPA (risque relatif ajusté= 1.53; IC 95%: 1.04- 2.25). La MG de BPA ajustée par la densité spécifique dans notre étude MIREC (0.90 µg/l) était inférieure à celle de Cantonwine et al. (2016) (1.34 µg/l) ce qui peut expliquer au moins partiellement les résultats discordants entre les études. Dans la population générale, nos résultats étaient cohérents avec ceux de Shiue (2014a) et de Shiue and Hristova (2014) qui n'ont pas trouvé d'association entre les concentrations urinaires du BPA et la TA élevée.

TCS

Les niveaux médians du TCS n'étaient pas associés au risque de l'hypertension gestationnelle

(aOR pour le troisième tertile (> 32.60 µg/l) = 1.41; 95% IC : 0.83, 2.37) ou de la prééclampsie (aOR pour le troisième tertile = 0.68; 95% IC : 0.31, 1.48). À notre connaissance, il n'y a pas d'autre étude chez les femmes enceintes examinant l'association entre l'exposition au TCS et les désordres hypertensifs de la grossesse ou la TA élevée. Dans la population générale, nous n'avons trouvé que deux études (Shiue 2014b; Shiue et Hristova 2014). Les deux études n'ont trouvé aucune association entre le TCS et la TA élevée. Un résumé des résultats significatifs des 3 articles est présenté dans le tableau 36.

Tableau 17. Résumé des principaux résultats significatifs des 3 articles.

Exposition	Hypertension gestationnelle globale[a]	Hypertension gestationnelle[b]	Prééclampsie[b]	TAM
	OR (95% IC) ajusté	OR (95% IC) ajusté	OR (95% IC) ajusté	Beta (95% IC) ajusté
Les métaux et les désordres hypertensifs de la grossesse (article 1)				
Métaux sanguins mesurés au 1er trimestre				
Analyse individuelle				
Mn (tertiles) <7.69 7.69 - 9.89 > 9.89	 Référence 0.98 (0.67, 1.42) 0.68 (0.46, 0.99)*	 Référence 0.95 (0.61, 1.49) 0.77 (0.49, 1.21)	 Référence 1.03 (0.55, 1.92) 0.48 (0.23, 0.98)*	
Stratification par le sexe de l'enfant				
Mn (tertiles) : enfant mâle <7.69 7.69 - 9.89 > 9.89	 Référence 0.73 (0.45, 1.19) 0.41 (0.24, 0.70)*	 Référence 0.68 (0.38, 1.21) 0.41 (0.22, 0.75)*	 Référence 0.86 (0.37, 2.03) 0.42 (0.16, 1.10)	

As (tertiles) ≤ 0.60 0.61 - 0.97 > 0.97	 Référence 1.85 (1.02, 3.36)* 1.76 (0.96, 3.22)	 Référence 1.54 (0.83, 2.86) 1.27 (0.67, 2.40)	 Référence 1.67 (0.60, 4.66) 2.11 (0.79, 5.67)	
Analyse avec les autres métaux				
As (tertiles) < 0.60 0.60 - 0.97 > 0.97	 Référence 1.14 (0.74, 1.74) 1.16 (0.74, 1.84)	 Référence 1.07 (0.64, 1.77) 1.00 (0.57, 1.74)	 Référence 1.94 (0.83, 4.55) 2.75 (1.13, 6.73)*	
Mn (forme continue)	0.93 (0.87, 0.99)*	0.93 (0.86, 1.00)	0.92 (0.82, 1.04)	
Métaux sanguins mesurés au 3ième trimestre				
Analyse individuelle				
As (forme continue)	1.01 (0.91, 1.13)	0.77 (0.55, 1.08)	1.16 (1.03, 1.30)*	
Hg (forme continue)	0.69 (0.50, 0.96)*	0.67 (0.44, 1.04)	0.94 (0.55, 1.60)	
Hg (tertiles) <0.36 0.36 - 0.82 > 0.82	 Référence 0.75 (0.50, 1.14) 0.62 (0.39, 0.98)*	 Référence 0.75 (0.45, 1.26) 0.67 (0.37, 1.21)	 Référence 1.03 (0.48, 2.21) 0.74 (0.29, 1.91)	
Analyse avec les autres métaux				
Hg (forme continue)	0.66 (0.47, 0.93)*	0.73 (0.47, 1.15)	0.79 (0.43, 1.46)	
Mesures concurrentes avec les métaux sanguins mesurés au 1er et au 3ième trimestre				
As sanguin (ln)[c]				-0.59 (-0.92, -0.26)*
Mn (ln)[c]				1.52 (0.80, 2.24)*
Mesures concurrentes avec les espèces de l'arsenic urinaire mesurées au 1er trimestre				
DMA (ln)[d]				-1.00 (-1.52, -0.48)*

Statut des amalgames dentaires et l'hypertension gestationnelle globale (article 2)				
Nombre des amalgames dentaires	NS			
Remplacement des amalgames dentaires	NS			
Les phénoles (article 3)				
BPA		NS	NS	
TCS		NS	NS	

OR = odds ratio (rapport de côte). IC = intervalle de confiance. Ln = logarithme naturel. DMA = acide diméthylarsinique. BPA = bisphénol A. TCS = triclosan.
[a]Régression logistique entre les métaux et l'hypertension gestationnelle globale
[b]Régression multinomiale entre les métaux et l'hypertension gestationnelle et la prééclampsie
[c]Équation d'estimation généralisée linéaire entre les métaux sanguins et la TA
[d]Régression linéaire entre les espèces de l'arsenic urinaire et la TA
[*]$p < 0.05$

8.2 Commentaires sur la validité et les forces de l'étude

8.2.1 Validité interne et externe des études

La présence de biais dans toute étude peut engendrer un problème de validité interne. La présence de bias de sélection, d'information ou la présence de confusion peut avoir entachée les résultats.

-**Biais d'information** : comme la mesure n'est jamais parfaite, il y a la possibilité de biais d'information non-différentielle. Par contre, plusieurs aspects limitent la possibilité d'un tel biais. Par exemple, une bonne qualité de l'information est attendue concernant les grossesses passées car elles sont généralement documentées et cela permet aux participantes de se rappeler facilement ces expériences. En plus, dans l'étude MIREC le temps de mémoire requis pour les questions est généralement court (3 mois au maximum, à part les antécédents des pathologies

médicales). Pour le remplacement des amalgames dentaires, le temps de rappel ne dépassait pas un an. Aussi, les analyses ont limité la présence de tel biais avec la catégorisation des variables. Par exemple, pour les amalgames dentaires, le fait d'avoir effectué une catégorisation binaire (non et oui), permet de minimiser l'effet du biais de rappel. Les femmes n'ont eu qu'à se rappeler si elles avaient eu ou non des remplacements des amalgames dentaires et non pas le nombre précis. Dans le pire des scénarios, un biais d'information non-différentielle (aléatoire) créerait une sous-estimation des effets ou les faire tendre vers la nulle.

Il y a également très peu de chance que des biais d'information différentielle soient présents dans cette étude puisque toutes les mesures des tests sur les métaux, le BPA, le TCS ainsi que l'exposition relative aux amalgames dentaires ont été fait à l'aveugle, c'est-à-dire sans connaissance du statut de l'issue.

Une seule mesure par personne du BPA et du TCS a été effectuée au premier trimestre de la grossesse. Les données semblent être le reflet de l'exposition au moment de la prise de l'échantillon à cause de la courte demi-vie des phénols analysés. La demi-vie de BPA et de TCS sont respectivement de <2 h (WHO 2010) et de 10.8h (Queckenberg et al. 2010). Le fait que la mesure de BPA et de TCS soit effectuée une seule fois et que leur demi-vie soit courte, cela pourrait conduire à une erreur de classification des participantes (risque élevé de classer des femmes non exposées aux phénoles alors qu'elles le sont).

-**Biais de confusion** : une analyse rigoureuse et conservatrice des potentielles covariables a permis d'identifier de façon exhaustive les variables de confusion. Les analyses multivariées ont permis de contrôler ce biais. Il n'est pas exclu cependant, que de la confusion résiduelle soit présente ou encore qu'une variable de confusion non considérée ou ayant des données

manquantes (le gain de poids durant la grossesse par exemple) dans cette étude puisse engendrer un tel biais. Par ailleurs, il existe différente façon de traiter la confusion comme par exemple l'utilisation du graphe orienté acyclique (DAG : directed acyclic graph), les scores de propension, la pondération inverse ou encore la détermination *a priori* des variables à contrôler. Il est possible que notre méthode d'ajustement (méthode du changement dans les estimés) ait produit par exemple un sur-ajustement.

-**Biais de sélection** : Ce biais est assez peu probable dans une étude de cohorte comme on ne connaît pas le statut de l'exposition ni même de l'issue au recrutement. Par contre, notre étude n'a retenu que 1909 participantes qui avaient accouché de bébés vivants. Les femmes qui avaient eu des avortements ou des morts fœtales (n = 74) ont été exclues de l'analyse. Étant donné que les causes des avortements et des morts fœtales sont multifactorielles, il serait difficile d'imaginer que les pertes soient associées autant à l'issue qu'à l'exposition (répartition non aléatoire des pertes). Il est fort probable qu'il n'y ait pas de biais de sélection malgré que la possibilité d'un biais non identifié soit toujours possible.

Par ailleurs, les modèles de régression multivariés pouvaient inclure des variables de confusion avec des données manquantes (généralement moins de 5%). Les variables de confusion potentielles ont été considérées en continue pour obtenir plus de puisasnce. Par contre, la non considération des données manquantes pouvait conduire à l'exclusion de femmes dans les modèles. Compte tenu de la faible proportion des données manquantes, il ne semble pas plausible de croire à un biais de sélection par rapport à ces modèles. Par ailleurs, les modèles univariés et les modèles multivariés n'ont pas montré de grandes différences dans les risques relatifs (la confusion contrôlée semblait faible puisque que les risques relatifs bruts et ceux ajustés n'ont pas

montré de grandes différences). L'impact de la considération des données manquantes n'a donc pas été important.

-**Validité externe** : Les participantes de l'étude MIREC présentent des caractéristiques socio-démographiques qui les rendent différentes de la population générale canadienne (Arbuckle et al. 2013). La majorité des participantes étaient caucasiennes, universitaires, non-fumeuses et avaient un revenu familial de plus de 90,000 $ CAN. Ces caractéristiques de notre population d'étude peuvent constituer une limite à la validité externe de notre étude et réduire le spectre de généralisation de nos résultats. Ce qui implique une prudence dans la généralisation de nos résultats à des populations non-caucasiennes et socio-économiquement différentes. Par ailleurs les centres tertiaires comme le centre hospitalier universitaire de Sainte-Justine incluent généralement un bassin plus important de femmes ayant des grossesses à risques. Il y a une possibilité que cela affecte aussi la généralisation des résultats.

8.2.2 Forces des études

Les principales forces de ce projet de recherche incluent en tout premier lieu son design (étude de cohorte prospective) et la grande taille de l'échantillon (n = 1909). Aussi les mesures des expositions ont été réalisées dans le laboratoire de référence de Québec (INSPQ). Les mesures des différentes issues ont été standardisées dans tous les centres affiliés de l'étude MIREC. Ces caractéristiques augmentent la possibilité de résultats valides. En plus, le caractère multicentrique de l'étude permet de dresser un profil d'exposition pan-canadien et entraîne une amélioration du potentiel de généralisation des résultats. À notre connaissance, nous semblons être les premiers à étudier les associations entre les amalgames dentaires ou le TCS et les désordres hypertensifs de la grossesse.

8.3 Pertinence pour la santé publique et orientations futures pour la recherche

L'étude MIREC est une étude pan-canadienne qui offre une banque de données exceptionnelles sur les différents contaminants environnementaux qui peuvent être associés à la survenue des désordres hypertensifs de la grossesse. Les résultats de nos études permettront de mieux comprendre la portée de ces contaminants sur la santé de la femme enceinte, en particulier les désordres hypertensifs de la grossesse, et d'orienter les politiques de santé publique pour limiter les expositions à ces contaminants. Par contre, malgré qu'on n'ait pas trouvé d'association entre les amalgames dentaires, le BPA ou le TCS et les désordres hypertensifs de la grossesse, il est préférable que le principe de précaution soit pris en compte dans les interventions de santé publique avec une limitation des sources d'exposition chez les femmes enceintes. Ce principe est déjà mis en place dans certains pays comme le Canada et la Suède dans le cas des amalgames dentaires à base de mercure (Health Canada 2009).

Les faibles niveaux des contaminants mesurés dans le sang comme dans l'urine dans l'étude MIREC par rapport à ceux rapportés par certaines études (Farzan et al. 2015; Kosanovic and Jokanovic 2007; Magri et al. 2003; Motawei et al. 2013; Shankar and Teppala 2012; Wells et al. 2017) envoient le message que les politiques canadiennes par rapport à la réduction de l'exposition aux contaminants chimiques environnementaux fonctionnent relativement bien. Par contre, les niveaux de l'As étaient associés à un risque très élevé de prééclampsie ce qui veut dire que malgré les acquis obtenus, de nouvelles pistes d'interventions doivent être explorées y compris la revue à la baisse des niveaux dits "valeurs limites". Nous suggérons aussi la réalisation d'études de cohortes additionnelles pour confirmer nos résultats dans des populations

ayant des niveaux d'exposition similaires à ceux de l'étude MIREC. En plus, il serait nécessaire de faire des études sur les liens entre les amalgames dentaires, le Hg, le BPA ou le TCS et les désordres hypertensifs de la grossesse ou le risque de TA élevée pour mieux clarifier la nature de leur association.

Chapitre 9 - Conclusion

Des concentrations sanguines plus élevées de Mn et de Hg ont été associées à un risque plus faible de désordres hypertensifs de la grossesse, tandis que l'As sanguin était associé à un risque élevé. Ces associations n'ont pas changé lorsque tous les métaux ont été inclus dans un même modèle. Lorsque nous avons analysé les associations entre les concentrations sanguines des métaux et les mesures concurrentes de la TA, nous avons constaté que la concentration sanguine de l'As (et celle de DMA dans l'urine) était inversement associée à la TA, alors que le Mn sanguin était positivement associé à une TA plus élevée. Le Mn sanguin a été associé à un risque plus faible d'hypertension gestationelle globale et d'hypertension gestationnelle chez les femmes porteuses de fœtus masculin. Les amalgames dentaires, le BPA ou le TCS n'étaient pas associés aux désordres hypertensifs de la grossesse.

Ces résultats permettront de comprendre la portée des contaminants étudiés sur les désordres hypertensifs de la grossesse, et de mieux orienter les politiques de santé publique pour limiter les expositions à ces contaminants. Aussi d'autres études sont nécessaires pour déterminer si ces résultats peuvent être reproduits dans d'autres populations similaires avec des niveaux d'exposition relativement faibles.

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Annexe - Matériel Supplémentaire

Annexe 1 : Autre classification des désordres hypertensifs de la grossesse

Tableau 18. Classification des désordres hypertensifs de la grossesse selon le NHBPEP (National High Blood Pressure Education Program) de 2000 (Cunningham et al. 2010; Lenfant 2001).

Selon cette classification, les désordres hypertensifs de la grossesse peuvent être catégorisés en quatre groupes : l'hypertension gestationnelle, l'hypertension chronique, la prééclampsie/ éclampsie et la prééclampsie surajoutée à l'hypertension chronique. Chacun de ces groupes présente les caractéristiques particulières suivantes.

L'hypertension gestationnelle (ou transitoire ou tardive): est définie comme une TA ≥ 140/90 qui apparaît pour la première fois à partir de la 20^{ième} semaine de gestation et sans protéinurie. Elle peut être caractérisée sous deux formes : (1) hypertension transitoire de la grossesse si la prééclampsie n'est pas présente au moment de l'accouchement et la tension artérielle revient à la normale 12 semaines après l'accouchement ou (2) l'hypertension chronique si l'élévation persiste.
L'hypertension chronique: se définit comme une augmentation de la TA ≥ 140/90 mmHg qui apparaît avant la grossesse ou avant la 20^{ième} semaine de gestation. Elle peut être aussi une hypertension artérielle diagnostiquée après les 20 semaines de gestation qui persiste après le postpartum.
La prééclampsie/éclampsie: la prééclampsie se caractérise par l'apparition d'une TA ≥140/90 mmHg à partir des vingt semaines de gestation. Elle peut s'accompagner de protéinurie qui est définie comme une excrétion urinaire de protéine de ≥ 0.3 g dans un échantillon d'urine de 24 h. Elle est corrélée à une détermination aléatoire de protéine ≥ 30 mg/dl (1 + à la bandelette urinaire) à l'absence d'infection. La protéinurie doit avoir lieu pour la première fois pendant la grossesse et disparaître après l'accouchement. La prééclampsie est suspectée en présence de la TA élevée accompagnée de céphalées, des troubles de la vision, douleurs abdominales, une perturbation des tests de laboratoire comme faible nombre de plaquettes et les enzymes hépatiques anormales. La présence de convulsion sans aucune autre cause, souligne l'apparition de l'éclampsie. La prééclampsie peut présenter TA ≥160/110 mmHg comme signe de gravité.
La prééclampsie surajoutée à l'hypertension chronique: se définit comme l'augmentation subite de la TA ≥140/90 mmHg chez la femme dont l'hypertension a été antérieurement bien contrôlée. Elle peut apparaître chez la femme hypertendue sans protéinurie avant les 20 semaines mais qui en débute une nouvelle ou avec protéinurie avant les 20 semaines. Elle est aussi caractérisée par une augmentation subite de la protéinurie, la trombocitopénie (faible nombre de plaquettes) et les enzymes hépatiques anormalement élevées. Cette catégorie a le pire pronostic pour la mère et le foetus par rapport aux autres.

Annexe 2 : Résumé des études selon la littérature

Tableau 19. Résumé des études sur l'association des métaux, amalgames dentaires, bisphénol A (BPA) ou triclosan (TCS) et l'hypertension artérielle chez les femmes enceintes ou dans la population générale

Exposition	Auteurs	Pays	Taille	Devis	Femmes enceintes	Population générale	Résultats : risque relatif / beta ou p-value de la comparaison	Niveaux	Strati-fication
As	Maduray et al. 2017	Sud-afrique	66 femmes enceintes	Cas-témoins			NS	Prééclampsie = 0.06 ± 0.0 (0.06, 0.06) µg/l) contrôle=0.49 ± 0.0 (0.01, 0.13) µg/l, p = 0.81	
	Sandoval-Carrillo et al. 2016	Mexique	306	Cas-témoins	✓		NS	Prééclampsie = 7.1µg/l (5.74) Contrôle = 6.78 µg/l (3.48), p = 0.428	
	Hall et al. 2017	Chilie	1266	Cas-témoins		✓	-Positif et significatif pour l'hypertension: OR = 1.65 (1.18, 2.32) pour la plus haute catégorie		
	Abhyankar et al. 2012	monde	20000	Revue systématique (11 études transversales (1995-2011)		✓	-Positif et significatif pour l'hypertension : OR (pooled)= 1.27 (1.09, 1.47) pour la plus haute catégorie		
	Yu et al. 2017	Chine	398 femmes	Cas-témoins		✓	-Positif et significatif pour l'hypertension : OR = 2.55 (1.55, 4.20)		
	Mordukhovich et al. 2012	USA	639 hommes	Transversal		✓	-Positif et significatif pour TA : TAS : beta = 1.43 (0.34, 2.51), p<0.05 Négatif TAD : beta = 0.63 (0.05, 1.21), p<0.05		
	Afridi et al. 2015	Irlande	243	Cas-témoins		✓	-Positif et significatif Niveaux élevés significativement chez les hypertendus par rapport aux		✓

							contrôles et p<0.001 si fumeurs		
Espèces d'As urinaire	Farzan et al. 2015	USA	514	Cohorte	✓		-Positif et significatif pour TA : -Chaque augmentation d' As de 5µg/l TAS = 0.15 mmHg (0.02, 0.29) et pression d'impulsion (0.14mmHg (0.02, 0.25) par mois		
	Jones et al. 2011	USA	4167	Transversal (NHANES 2003-2008)		✓	NS OR pour l'hypertension : total As = 0.98 (0.86, 1.11) total As moins arsenobetaine = 1.03 (0.94, 1.14) DMA = 1.11 (0.99, 1.24)		
	Li et al. 2013	Chine	604	Transversal		✓	-Positif et significatif pour l'hypertension: OR (MMA en µg/g Cr) = 1.693 (1.028, 2.787) -NS pour DMA, As inorganique (en %)		
	Shiue 2014a	USA		Transversal (NHANES 2009-2010)		✓	Positif et significatif pour TA: OR (DMA) = 1.35 (1.06, 1.73)		
	Shiue 2014b	USA	9756	Transversal (NHANES 2011-2012)		✓	Positif et significatif pour TA: OR (DMA) = 1.42 (1.12, 1.79)		
Pb	Magri et al. 2003	Malte	143	Transversal	✓		Positif et significatif	Les niveaux moyens de Pb significativement plus élevés chez les femmes avec l'hypertension gestationnelle (9.6 ± 6 µg/dl) par rapport aux contrôles (5.8 ± 3 µg/dl), p = 0.002	
	Rabinowitz et al. 1987	USA	3851	Transversal	✓		Positif et significatif pour TA : TAS : r = 0.081, p <0.001 TAD : r = 0.051, p = 0.002		
	Motawei et al. 2013	Egypte	140	Transversal	✓		Positif et significatif	Prééclampsie = 37.68 ± 9.17 µg/dl contrôle = 14.5 ± 3.18 µg/dl	

	Ikechukwu et al. 2012	Nigeria	209	Cas-témoins	✓		Positif et significatif	Prééclampsie = 60.2 ± 12.8 µg/dl contrôle = 26.3 ± 8.0 µg/dl	
	Sowers et al. 2002	USA	705	Cohorte	✓		Positif et significatif p < 0.03	1.2 µg/dl (±0.03) pour prééclampsie	
	Wells et al. 2011	USA	285	Cohorte	✓		Positif et significatif pour TA : TAS: beta = 6.87 (1.51, 12.21) TAD: beta = 4.40 (0.21, 8.59)		
	Jameil 2014	Arabie Saoudite	120	Cas-temoins	✓		Positif et significatif Prééclampsie = 27.18 ± 2.13 µg/dl dans le sérum contrôle = 18.23 ± 2.34 µg/dl, p <O.05		
	Vigeh et al. 2006	Iran	396	Cas-témoins	✓		Positif et significatif OR = 12.96 (1.57, 107.03)	Prééclampsie = 4.30±2.49 µg/dl) Contrôles = 3.52±2.09 µg/dl, p<0.05	
	Maduray et al. 2017	Sud-afrique	66 femmes enceintes	Cas-témoins	✓		NS	Prééclampsie = 0.20 ± 0.17 (0.04, 5.49) µg/l Contrôle = 0.16 ± 0.21 (0.0, 3.0) µg/l, p = 0.22	
	Yazbeck et al. 2009	France	1017	Cohorte	✓		-Positif et significatif pour l'hypertension gestationnelle : OR = 3.3 (1.1, 9.7)		
	Bushnik et al. 2014	Canada	4550	Transversal (CHMS 2007-2011)		✓	-Positif pour TA: Hommes : TAS: beta = 2.17 (-0.08, 4.42), p = 0.058 TAD : beta = 2.36 (0.94, 3.79), p = 0.002 -NS chez les femmes TAS : beta = 0.76 (-2.72, 4.24), p = 0.656 TAD : beta = 1.43 (-0.51, 3.38), p = 0.142 Hypertension : beta (homme) = -6.37 (-15.02, 2.29), p = 0.142		✓

							Beta (femme) = -4.18 (-8.78, 0.42), p = 0.073		
	Mordukhovich et al. 2012	USA	639 hommes	transversal		✓	NS pour TA : TAS : beta = –0.22 (–0.93, 0.50) , p>0.05 TAD : beta = 0.27 (–0.11, 0.65), p>0.05		
	González-Muñoz et al. 2010	Espagne	26 femmes menaupos ées	Cas-témoins		✓	NS	Hypertendues = 1.26 (0.66, 1.94) Non-hypertendues = 1.04 (0.71, 2.31), p>0.05	
	Afridi et al. 2015	Irlande	243	Cas-témoins		✓	Positif et significatif	Niveaux élevés significativement chez les hypertendus par rapport aux contrôles et p<0.001 si fumeurs	✓
	Lee et al. 2016	Corée du sud	11979	Transversal		✓	Positif et significatif pour TA : TAS : beta = 0.73 (0.09-1.36), p<0.05 TAD : beta = 0.71 (0.29-1.13), p<0.05		
Cd	Kosanovic and Jokanovic 2007	Belgrade	60	Cas-témoins	✓		Positif et significatif	Hypertendue gestationnelle fumeuse: 1.9 ± 0.6 µg /l Normotendue et non fumeuse: 0.8 ± 0. 3 µg /l Hypertendue gestationnelle et non fumeuse : 1.3 ± 0.1 µg/l, p = 0.001	✓
	Kosanovic et al. 2002 (similaire à la précédente)	Belgrade, Yougoslavie	60	Cas-témoins	✓		Positif et significatif	Hypertendue gestationnelle fumeuse: 1.9 ± 0.6 µg /l Normotendues et non fumeuse: 0.8 ± 0. 3 µg /l Hypertendue	✓

								gestationnelle et non fumeuse : 1.3 ± 0.1 µg/l, p = 0.001	
	Yazbeck et al. 2009	France	1017	Cohorte	✓		NS		
	Laine et al. 2015	USA	172	Cas-témoins niché	✓		-Positif et significatif pour la prééclampsie -OR = 1.5 (1.1, 2.2)		
	Lazebnik et al. 1989	USA	43 hypertendues et leurs contrôles	Cas-témoins	✓		NS	Pas de différence entre les niveaux de Cd dans le sang et dans le placenta pour les normotendues et les hypertendues	
	Eum et al. 2008	Corée	1902	Transversal (KNHANES)		✓	Positif et significatif pour l'hypertension OR = 1.51 (1.13, 2.05)		
	Al-Saleh et al. 2006	Arabie saoudite	185	Cas-témoins		✓	NS p = 0.098	Hypertendues = 0.874 ± 0.995 µg/l Contrôles = 0.785 ± 0.665 µg/l	
	Shiue et Hristova 2014	USA	20293	Transversal (NHANES 2009-2012)		✓	NS pour TA : Hommes: OR = 0.99 (0.75, 1.30), p = 0.924 Femmes: OR = 1.13 (0.86, 1.48), p = 0.375		✓
	Mordukhovich et al. 2012	USA	639 hommes	Transversal		✓	NS pour TA : TAS : beta = 0.21 (-0.13, 0.56), p>0.05 TAD : beta = 0.08 (-0.10, 0.27), p>0.05		
	Tellez-Plaza et al. 2008	USA	10991	Transversal (NHANES 1999–2004)		✓	-Positif et significatif pour TA : TAS : beta = 1.36 mmHg TAD : beta = 1.68 mmHg -chez les fumeurs actuels TAS : beta = 0.02 mmHg TAD : beta = 0.69 mmHg -chez les non-fumeurs TAS : beta = 2.35 mmHg TAD : beta = 3.27 mmHg		✓
	Afridi et al. 2013	Irlande	243	Cas-témoins		✓	Positif et significatif	Niveaux de Cd significativement	✓

								élevés chez les hypertendus que chez les contrôles surtout s'ils sont fumeurs (p<0.001)	
	Lee et al. 2016	Corée du sud	11979	Transversal		✓	Positif et significatif pour TA : TAS : beta = 1.55 (1.10-2.00), p<0.05 TAD : beta = 0.93 (0.64-1.23), p<0.05		
Hg	Wells et al. 2017	USA	263	Transversal	✓		-Positif et significatif pour TA: Méthylmercure : TAS : beta = 2.83 (0.17, 5.50), p<0.05 pression d'impulsion: beta = 2.99 (0.91, 5.08), p<0.05 -Négatif et significatif: Hg inorganique : pression d'impulsion beta = -2.51 (-4.49, -0.53), p<0.05 -Négatif et NS Hg inorganique : TAS: beta = -1.18 (-3.72, 1.35), p>0.05	Méthylmercure (95% IC) = 0.95 µg/l (0.84, 1.07) Hg inorganique = 0.13 µg/l (0.10, 0.17)	
	Pan et al. 2007	Chine	2148 femmes et 2044 contrôles	Méta-analyse (14 études en chinois 1989-2006)	✓	✓	Positif et significatif hypertension gestationnelle : RR = 2.17 (1.32, 3.57)		
	Park et al. 2013	USA	2201	Transversal (NHANES 2003-2006)		✓	-Négatif et significatif pour l'hypertension: urine: OR = 0.87 (0.78, 0.99) -Négatif et NS sang: OR = 0.94 (0.87, 1.01)		
	Nielsen et al. 2012	Groenland	1861	Transversal		✓	-Négatif et significatif pour TA: hommes: TAD: beta = -0.04, p = 0.001 OR = 0.99 (0.98, 0.99) -Négatif et NS: TAS (homme): beta = -0.02, p = 0.06 femmes: TAD: beta = -0.01, p = 0.19 TAS: beta = -0.003, p = 0.77		✓

							OR = 1.00 (0.99, 1.01), p = 0.65		
	Mozaffarian et al. 2012	USA	6045	Cas-témoins niché		✓	Négatif et NS pour l'hypertension : hazard ratio: -femmes = 0.96 (0.84, 1.09) -hommes = 0.82 (0.62, 1.08) -les deux = 0.94 (0.84, 1.06)		✓
	Goodrich et al. 2013	USA	262	Cohorte		✓	-Positif et significatif pour TA: Chéveux :TAD : beta = 2.76, p = 0.02 -Négatif et significatif Urine: TAS: beta = -1.8, p = 0.04		
	Pedersen et al. 2005	Groenland Danemark	186	Transversal		✓	-Négatif et significatif pour TA: TAD: beta = -3.112, p = 0.014 -Positif et NS TAS: beta = 0.516, p = 0.749		
	Mordukhovich et al. 2012	USA	639 hommes	Transversal		✓	Négatif et NS pour TA: TAS: beta = -0.76 (-2.68, 1.16) TAD: beta = -0.27 (-1.32, 0.78)		
	Bautista et al. 2009	Wisconsin	101	Transversal (étude pilote)		✓	-Positif et significatif pour l'hypertension: Cheveux: OR = 4.19 (1.28, 13.76), p = 0.02 -Positif et NS Sang: OR = 1.93 (0.66, 5.65), p = 0.23		
	Fillion et al. 2006	Brésil	251	Transversal		✓	-Positif et significatif pour TA : TAS (≥130 mmHg) : OR = 2.91 (1.26–7.28) -Positif et NS : TAD (≥ 90 mmHg) : OR = 2.29 (0.95–6.06)		✓
	Vupputuri et al. 2005	USA	1240 femmes	Tansversal (NHANES 1999-2000)		✓	Chaque augmentation de 1.3 µg/l : - Positif pour TA : non consommateurs de poissons :		✓

							TAS : beta = 1.83 (0.36, 3.30), p<0.05 TAD = 0.61 (-1.58, 2.80), p>0.05 -Négatif et NS pour TA : consommateurs de poissons : TAS = -0.14 (-0.83, 0.55) DBP = -0.17 (-0.71, 0.37), p>0.05		
Mn	Vigeh et al. 2006	Iran	396	Cas-témoin	✓		Positif et significatif OR = 34.2 (1.81, 648.04)	Prééclampsie = 46.87±15.03µg/l Contrôle = 40.32±15.19 µg/l, p<0.05	
	Vigeh et al. 2013	Iran	364	Cohorte	✓		Positif et significatif pour l'hypertension gestationnelle : 1er trimestre : OR= 47.0 (4.0, 556.4) 2e trimestre: OR = 5.5 (1.1, 29.0)	Niveaux moyens au 1er trimestre = 18.6 µg/l et 2e trimestre = 18.9 µg/l	
	Sarwar et al. 2013	Bangladesh	108	Cas-témoins	✓		Négatif et significatif	Prééclampsie = 0.08 ± 0.02 µg/l Contrôle = 0.14 ± 0.02µg/l, p = 0.032	
	Al-Jameil et al. 2014	Arabie Saoudite	120	Cas-témoins	✓		Négatif et significatif	Prééclampsie = 0.072 ± 0.06µg/l Contrôle = 0.125 ±0.07µg/l p<0.001	
	Maduray et al. 2017	Sud-afrique	66	Cas-témoins	✓		Négatif et significatif	Prééclampsie = 0.02 ± 0.0 (0.01, 0.03) µg/l) contrôle = 0.03 ± 0.0 (0.0, 0.16) µg/l, p = 0.03	
	Yazbeck et al. 2009	France	1017	Cohorte	✓		NS pour l'hypertension gestationnelle		
	Mordukhovich et al. 2012	USA	639 hommes	Transversal		✓	Négatif et significatif pour TAM : TAS : beta = -1.09 (-2.08, -0.10), p<0.05 TAD : beta = -0.62 (-1.15, -0.09), p<0.05		
	Lee and Kim 2011	Corée du sud	1991	Transversal (KNHANES 2008)		✓	Positif et significatif pour l'hypertension : Femmes: OR = 1.828 (1.096–		✓

							3.048) Hommes: OR = 1.573 (1.083–2.284) Les deux: OR = 1.567 (1.167–2.103)		
	González-Muñoz et al. 2010	Espagne	26 femmes menaupos ées	Cas-témoins		✓	Négatif et significatif - TAS : r = -0.423, p = 0.031	Mesure dans les cheveux: Postménauposées hypertendues = 0.08 µg/g (0.06, 0.11) Contrôles = 0.16 µg/g (0.11, 0.37, p<0.05	
	Lee et al. 2015	Corée du sud	640	Transversal		✓	Négatif et significatif pour TA : TAS : r = -0.1303 chez les hommes, p <0.01 TAD : NS chez les hommes et les femmes		✓
	Kim and Choi 2013	Corée du sud	258	Cohorte		✓	-Négatif et significatif pour TA : TAS: r = -0.1323, p<0.05 -NS TAD: r = -0.1091 p>0.05		
BPA	Leclerc et al. 2014	Canada	58 (23 cas)	Cas-témoins	✓		NS Positif	-**Sérum maternel** : Prééclampsie = 2.80 µg/l (0.21-9.00) Contrôle = 3.00 µg/l (0.04-24.2),p = 0.67 -**Sérum fetal** Prééclampsie = 2.23 (0.21–16.4) Contrôle = 2.17 (0.12–7.75), p = 0.57 -**Placenta** Prééclampsie=9.40 µg/l (0.40–101) Contrôle = 3.00 µg/l (0.30-36.1), p = 0.04	
	Cantonwine et al. 2016	USA	482 (50 cas)	Cas-témoins niché	✓		Positif et significatif Hazard ratio pour prééclampsie = 1.53 (1.04,		

							2.25)		
	Shiue et Hristova 2014	USA	20 293	Transversal (NHANES 2009-2012)		✓	NS OR = 1.13 (0.98, 1.31)		
	Bae and Hong 2015	Corée du sud	60	Essai de croisement aléatoire		✓	En comparant à la boisson en verre -Positif et significatif pour TA : Différence relative de TAS : beta = 4.5101, p = 0.0187 -NS : TAD : beta = 0.7825, p = 0.4806 2 heures après la consommation de cannettes de boissons		
	Shiue 2014a	USA		Transversal (NHANES 2009-2010)		✓	NS pour TA: OR = 1.12 (0.93, 1.35)		
	Wang et al. 2015	Chine	3246	Transversal		✓	Négatif et significatif pour l'hypertension: OR = 0.61 (0.46, 0.80)		
	Bae et al. 2012	Corée du sud	521	Korean Elderly Environmental Panel Study (2008-2010)		✓	-NS pour l'hypertension : OR = 1.27 (0.85, 1.88) -Positif et significatif pour un sub-groupe sans antécédent de HTA (n = 258): OR = 2.35 (1.33, 4.17)		
	Aekplakorn et al. 2015	Thailande	2588	Transversal (TNHES IV 2009)		✓	-NS pour l'hypertension : Homnes: OR = 1.44 (0.99, 2.09) -Positif et significatif pour l'hypertension : Femmes: OR = 2.16 (1.31, 3.56)		✓
	Shankar and Teppala 2012	USA	1380	Transversal (NHNES 2003-2004)		✓	Positif et significatif pour l'hypertension: OR = 1.50 (1.12, 2.00)		
	Han and Hong 2016	Monde		Revue de la litterature		✓	Possible relation avec l'hypertension		
TCS	Shiue 2014b	USA	9756	Transversal (NHNES 2011-2012)		✓	NS pour TA: OR = 0.96 (0.90, 1.03), p = 0.308		
	Shiue and	USA	20 293	Transversal		✓	NS pour TA:		

	Hristova 2014			(NHANES 2009-2012)			OR = 0.98 (0.91, 1.05)		
Amalgames dentaires	Siblerud 1990	USA	101 femmes et hommes	Transversal		✓	Positif et signifcatif pour TA : -Comparaisons des sujets avec ou sans amalgames : augmentation de la TA, tachycardie, douleur de poitrine par exemple chez les porteurs d'amalgames dentaires -les hommes ont la TA plus élevée par rapport aux femmes		✓
	Ahlqwist et al. 1993	Suède	1462 femmes	Cohorte		✓	-Corrélation négative (valeur non précisée), p=0.03 pour infarctus du myocarde par exemple -NS RR = 0.23 (0.05, 1.18), pour infarctus du myocarde en comparant les sujets avec ≥20 surfaces d'amalgames dentaires à ceux qui en ont 0-4		
	Bengtsson et al. 2001	Suède	femmes	Cohorte		✓	Pas de différence dans l'incidence de l'infarctus du myocarde par exemple entre les grands exposés aux amalgames dentaires ou au Hg et les moins exposés		

HTA = hypertension artérielle. TA = tension artérielle. TAS = tension artérielle systolique. TAD = tension artérielle diastolique. NHANES = US National Health and Nutritional Examination Survey. TNHES = Thai National Health Examination Survey. KNHANES = Korean National Health and Nutritional Examination Survey. MMA = acide monométhylarsonique. DMA = acide diméthylarsinique.

Annexe 3 : Informations additionnelles concernant chaque article

a- Cadre conceptuel avec les variables spécifiques à chaque objectif

Figure 2. Objectif 1 (Metals and hypertensive disorders in pregnancy: MIREC study)

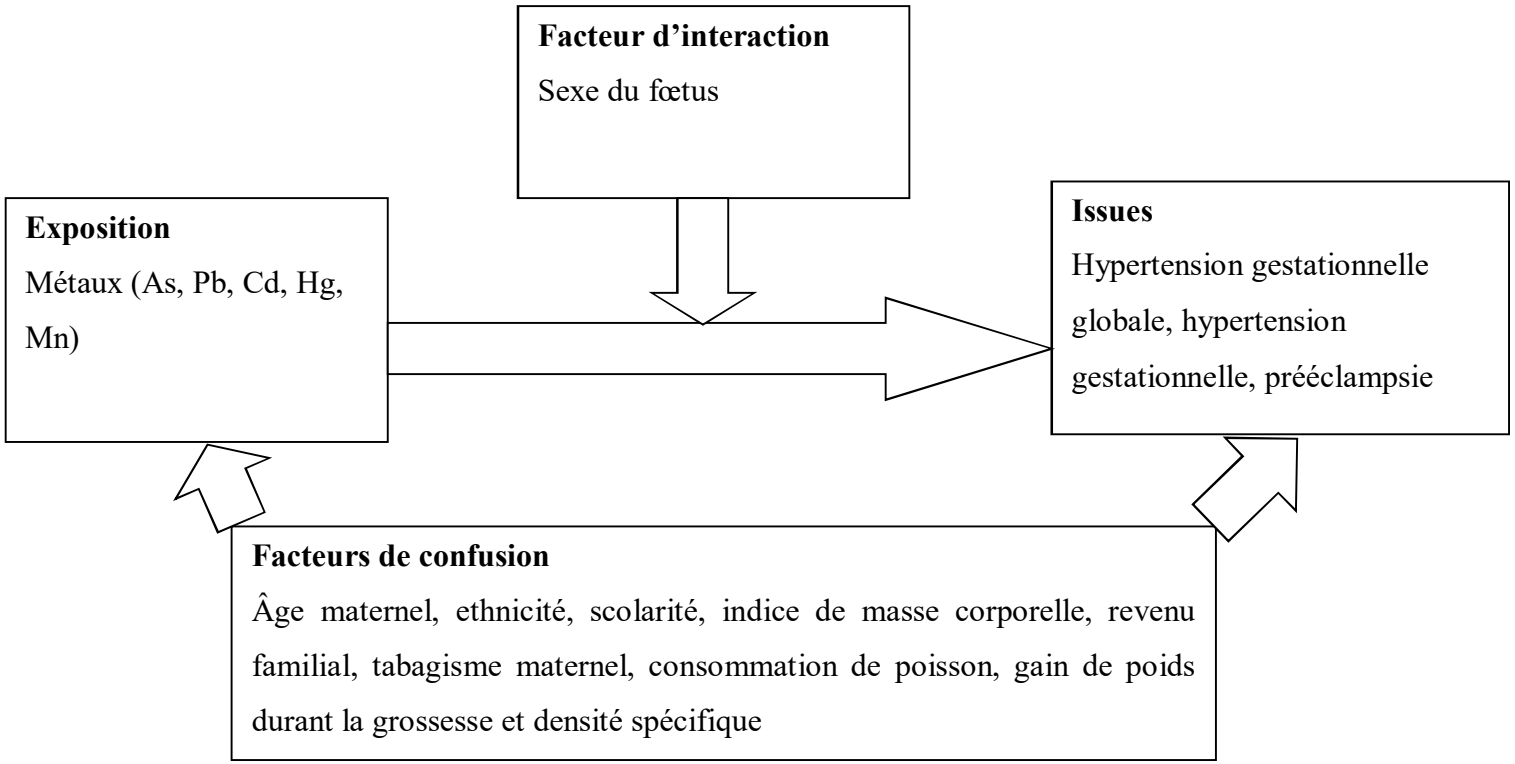


Figure 3. Objectif 2 : Dental amalgams and the risk of gestational hypertension in the MIREC Study

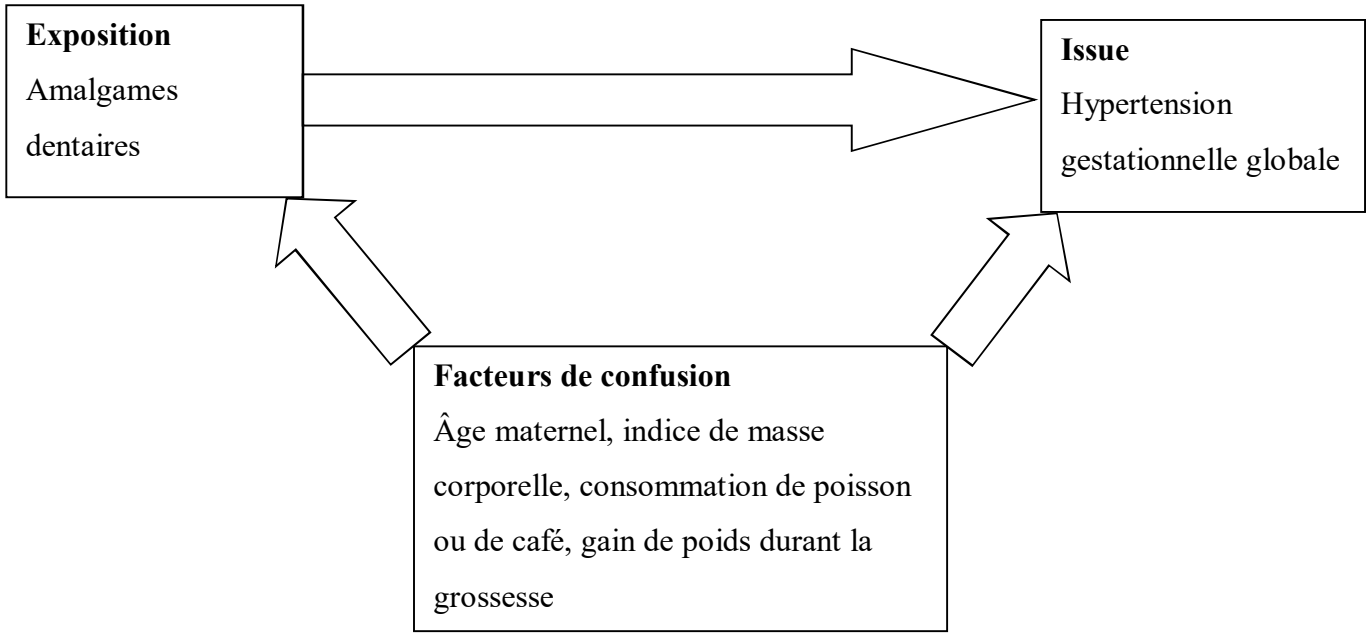
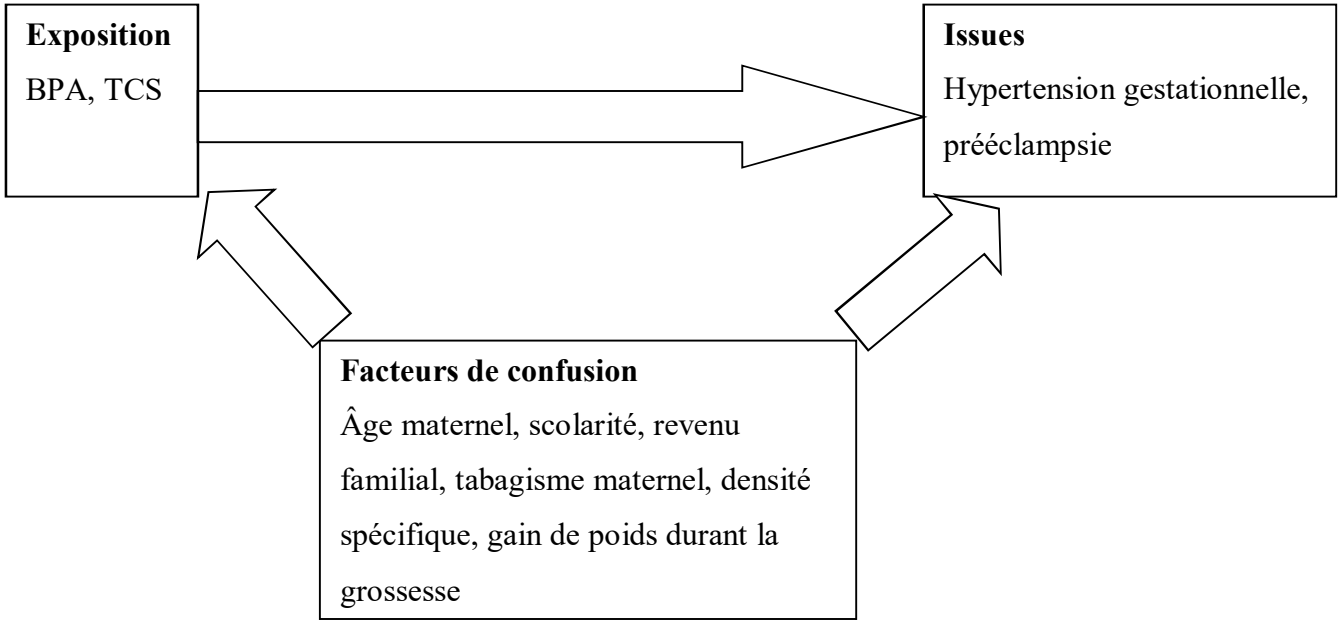


Figure 4. Objectif 3 : Association between exposure to bisphenol A or triclosan and gestational hypertension and preeclampsia: MIREC Study



Annexe 4 : Information supplémentaire sur la méthodologie de l'étude MIREC

4a- Cohort Profile: The Maternal-Infant Research on Environmental Chemicals Research Platform (voir l'article joint dans l'annexe 8)

4b- Protocole original de MIREC (voir copie jointe dans l'annexe 8)

4c- Questonnaires de MIREC (voir copie jointe dans l'annexe 8)

4d- Variables évaluées dans MIREC à chaque visite (voir tableau 40)

Tableau 20. Liste des variables évaluées dans MIREC à chaque visite

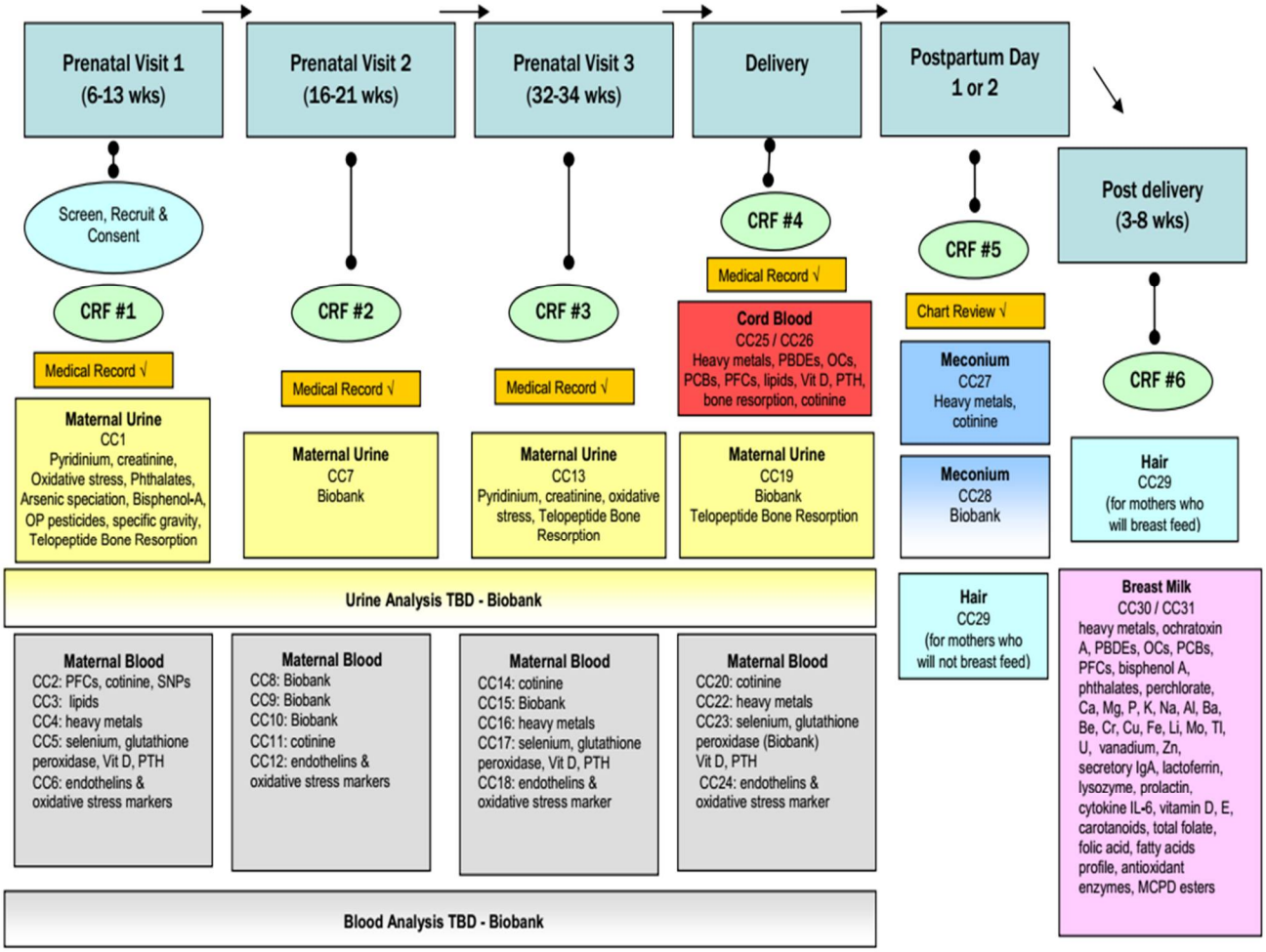
Measures	6-12 weeks	16-21 weeks	32-34 weeks	Delivery	1-2 days postnatal	2-10 weeks postnatal (breastmilk)
Demographics						
Maternal age, education, ethnicity, employment	□		□			
Paternal age, education, ethnicity, occupation	□					
Marital status, income	□					
Obstetric History						
Pregnancy history, time to preg	□					
Use of ART and contraceptive	□					
Pregnancy outcome	□	□	□	□		
Family Medical History	□					
Current Medications	□	□	□	□		
Nutritional Supplements	□	□				
Environmental Exposures						
Hobbies, work, home renovations, cooking ustentils	□		□			
Lifestyle						
Active and passive smoking	□		□			□
Alcohol consumption	□		□			□
Residential History						
Addresses, home characteristics	□		□			
Activities	□		□			
Diet						
Meat	□		□			
Fish	□		□			□
Food Frequency		□				□
Maternal anthropometric measures	□	□	□	□		□
Blood pressure	□	□	□	□		
Protein urine dipstick test	□	□	□			
Placenta weight				□		
Co-factor Biomonitoring						
pyridinium, creatinine, selenium, glutathione peroxidase	□		□	□		
oxidative stress, markers, endothelins	□		□	□		
minerals, vitamins, fatty acids, enzymes						□
Chemical Biomonitoring						
Metals	□		□	□	□	□
Plasticizers	□			□		□
Brominated flame retardants	□			□		□
Surface coatings	□			□		□
OP Pesticides	□			□		□
POPs (PCBs, OCs)	□			□		□
Cotinine	□	□	□	□	□	□
Genetic Polymorphisms		□				

ALAD, VDR (fokI, bsmI, apaI, taqI), APOE (1-4), HFE (C282Y, H63D), metallothionein		□				

4e- Collecte des données (prélèvements sanguins et urinaires) dans l'étude MIREC

Figure 5. Vue générale sur la collecte des données (prélèvements sanguins et urinaires) dans l'étude MIREC

Figure 2. Overview of measures taken at each collection phase



Annexe 5 : Résumé des covariables potentielles pour chaque article

Tableau 21. Résumé des variables de confusion potentielles correspondantes à chaque objectif de la thèse

Covariables potentielles	Premier objectif	Deuxième objectif	Troisième objectif
Tabagisme maternel	-jamais -cessé de fumer avant la grossesse -cessé de fumer pendant la grossesse -fume à l'heure actuelle	-non -oui	-non -oui
L'indice de masse corporel	-poids insuffisant -poids normal -surpoids -obésité	covariable continue	-poids insuffisant -poids normal -surpoids -obésité
La parité	-multipare -nullipare	-multipare -nullipare	-multipare -nullipare
L'âge maternel	covariable continue	covariable continue	18-31 32-35 36-49
L'ethnicité	- caucasienne - non-caucasienne	- caucasienne - non-caucasienne	- caucasienne - non- caucasienne
Le revenu familial	≤15,000 16,000-35,000 36,000-45,000 46,000-55,000 56,000-65,000 66,000-75,000 76,000-90,000 > 90,000 $ CAN	< 65,000 65,000 – 90,000 > 90,000 $ CAN	< 65,000 65,000 – 90,000 > 90,000 $ CAN
Consommation d'alcool pendant les 3 mois précédant la visite 1	-non -oui		
La consommation de poisson	covariable continue	covariable continue	
Le niveau de scolarité	-études supérieures -université -inférieur à l'université	-diplôme d'études supérieures -université de premier cycle -université -inférieur à l'université	-études supérieures -université -inférieur à l'université
Le gain de poids durant la grossesse	covariable continue	covariable continue	<12.40 kg 12.40-17.24 kg >17.24 kg

La consommation de café		-non -oui	
La grossesse multiple		-non -oui	
L'antécédent de maladie auto-immune		-non -oui	
La densité spécifique	covariable continue		covariable continue

Annexe 6 : Calcul de la puissance

OR MINIMAL DÉTECTABLE AVEC NOTRE TAILLE D'ÉCHANTILLON

Le calcul du OR minimal détectable a été estimé avec le logiciel du *National Center for Advancing Translational Sciences, National Institutes of Health, through UCSF-CTSI* disponible en ligne (http://www.sample-size.net/sample-size-proportions/).

P1 supposé plus grand que P0

α (two-tailed) =		*Threshold probability for rejecting the null hypothesis. Type I error rate.*
β =	0.200	*Probability of failing to reject the null hypothesis under the alternative hypothesis. Type II error rate.*
P_0 =	0.33	*Proportion in Group 0 (baseline proportion)*
N_1 =	187	*Number of subjects in Group 1*
N_0 =	1630	*Number of subjects in Group 0*

P_1 is expected to be ◉ greater than ○ less than the value of P_0.

This study has 80.0% power to detect a P_1 = 0.437
Risk ratio = P_1/P_0 = 1.324
Odds ratio = $(P_1/(1 - P_1))/(P_0/(1-P_0))$ = 1.576

P1 supposé plus petit que P0

α (two-tailed) =	0.050	*Threshold probability for rejecting the null hypothesis. Type I error rate.*
β =	0.200	*Probability of failing to reject the null hypothesis under the alternative hypothesis. Type II error rate.*
P_0 =	0.33	*Proportion in Group 0 (baseline proportion)*
N_1 =	187	*Number of subjects in Group 1*
N_0 =	1630	*Number of subjects in Group 0*

P_1 is expected to be greater than / less than the value of P_0.

This study has 80.0% power to detect a P_1 = 0.229
Risk ratio = P_1/P_0 = 0.694
Odds ratio = $(P_1/(1 - P_1))/(P_0/(1-P_0))$ = 0.603

Where P1 is Proportion in group 1

REFERENCES

Fleiss, JL, et al. *Statistical methods for rates and proportions.* Hoboken, NJ, J. Wiley; 2003. Page 79.

Formula used in this calculation

Given sample sizes n_0 and n_1, the proportion in the control group P_0, α, and β, find the proportion P_1 that the study has power $1 - \beta$ to identify. The effect size can be stated as a difference $P_1 - P_0$, a ratio P_1/P_0, or an odds ratio $(1 - P_1)/(1 - P_0)$.

Given a trial value of P_1, we can calculate z_β.

$$r = n_1/n_0$$
$$\overline{P} = \frac{P_0 + rP_1}{1 + r}$$
$$\delta = |P_1 - P_0|$$

$$z_\beta = \frac{\delta\sqrt{n_1 - (r+1)/\delta} - z_{\alpha/2}\sqrt{(r+1)\overline{P}(1-\overline{P})}}{\sqrt{rP_0(1-P_0) + P_1(1-P_1)}}$$

Try different values of P_1 until the desired z_β is returned.

Annexe 7 : Curruculum Vitae

1. Éducation

1.1 Formation universitaire

1. Automne 2012 à hiver 2018: Doctorat en Santé Publique. Université de Montréal. Québec (moyenne cumulative excellente).
2. Automne 2015 : Diplômée du microprogramme (2ième cycle) en recherche interdisciplinaire en santé de la reproduction. Université de Montréal. Québec (moyenne cumulative excellente).
3. Automne 2011 à Eté 2012: Maîtrise en Santé Communautaire. Université de Montréal (moyenne cumulative très bien).
4. 1998-2008 : Institut Supérieur de Sciences Médicales " Serafín Ruiz de Zárate Ruiz". Santa Clara (Cuba).
    a. 2004 : Médecin généraliste (mention excellente et major de la promotion)
    b. 2008 : Spécialiste en gynécologie et obstétrique (mention excellente).
    c. 2008 : Maîtrise en Soins Intégraux de la femme (mention excellente).
5. 1997-1998 : Préparation en langue espagnole. Université de Matanzas. Cuba.

1.2 Autres formations et compétences :

A. Bonne connaissance des logiciels informatiques tels que Word, Excel, PowerPoint et SPSS. Notions en STATA, SAS et R.
B. Février à Avril 2010: formation en médecine d'urgence. Clinique Ambroise Paré. Conakry. Guinée.
C. Entrainement à Santa Clara (Cuba) dans:
    a. Laparoscopie diagnostique et chirurgicale
    b. Chirurgie générale
    c. Chirurgie esthétique
    d. Échographie
    e. Génétique
    f. Informatique

2. Participation à des rencontres scientifiques internationales. Santa Clara. Cuba.

a. 2007 : Atelier International de Santé Reproductive
b. 2004 : Symposium d'Hypertension Artérielle

3. Présentations orales. Québec. Canada

a. Hiver 2018.

Titre : Association entre les métaux, les amalgames dentaires, les phénols et les désordres hypertensifs de la grossesse.
Auteur : Louopou Rosalie Camara
Lieu : École de Santé Publique. Avenue du Parc.

b. Hiver 2015.

Titre : Pour un Québec en santé : soutenons la recherche
Auteurs : Louopou Rosalie Camara, Joseph Niyibizi, Wafae bouhaddioui, Boukris Takoua

Lieu : École de Santé Publique. Avenue du Parc.

c. Automne 2013.
Titre: l'évaluation de l'association entre les métaux lourds et l'hypertension gestationnelle: la transdisciplinarité.
Auteur : Louopou Rosalie Camara
Lieu : Université de Montréal

d. Hiver 2013.
Titre: Quelques considérations sur les métaux lourds et l'hypertension gestationnelle.
Auteurs: Louopou Rosalie Camara et Victorin Capo-chichi Cocou
Lieu : École de Santé Publique. Avenue du Parc.

e. Hiver 2012.
Titre: Évaluation de l'effet de la vitamine D sur l'hypertension gestationnelle
Auteur : Louopou Rosalie Camara
Lieu : CHU Sainte-Justine

f. Automne 2011.
Titre: Tabagisme chez les jeunes
Auteurs: Rosalie, Djaouida, Sonia, Collins et Carlos
Lieu : Université de Montréal

4. Communications orales. Santa Clara. Cuba.

Présentations à hôpital "Mariana Grajales" de Santa Clara en 2008.

1. *Titre* : L'infection postpartum dans les accouchements transpelviens : analyse de 3 ans
   Auteur : Louopou Rosalie Camara
2. *Titre* : L'importance diagnostique et thérapeutique de l'utilisation de l'anse de diathermie dans le traitement de la néoplasie cervicale intraépithéliale.
   Auteur : Louopou Rosalie Camara

Congrès national:

1. XIII forum de science et technique de base 2eme partie. Cuba, juin 2000
   Titre: Facteurs de risque du retard de la croissance intra-utérine (Embryologie: matériel d'appui)
   Prix : Prix de participation

Congrès local:

1. Première journée étudiante sur les soins de santé primaires. Cuba, juin 2003
   Titre: les facteurs de risque des maladies cardiovasculaires
   Prix : Première mention
2. Journée scientifique étudiante départementale. Cuba, mai 2003
   Titre: Risk factors in cardiovascular diseases
   Prix: Premier prix
3. Forum étudiant sur les soins de santé primaires. Cuba, avril 2002
   Titre: Les facteurs de risque des maladies cardiovasculaires
   Prix : Prix d'encouragement
4. Forum scientifique étudiant de l'institut. Cuba en juillet 2000

Titre: les facteurs de risque des maladies cardiovasculaires
Prix : Prix de participation

5. Journée scientifique étudiante au niveau départemental. Cuba en mai 1999
Titre: Facteurs de risque de la croissance intra-utérine retardée (Embryologie: matériel d'appui)
Prix : Premier prix.

5. Publications

a. *Titre de l'article:* Metals and hypertensive disorders of pregnancy: MIREC Study
Statut de l'article : révision en cours du manuscrit à Santé Canada. Il sera soumis au journal Environmental Health Perspectives.

b. *Titre de l'article* : Dental amalgams and risk of gestational hypertension in the MIREC Study
Statut de l'article : le manuscrit est en correction à Santé Canada. L'article sera soumis au New England Journal of Medecine.

c. *Titre de l'article* : Association between maternal exposure to bisphenol A or triclosan and gestational hypertension and preeclampsia: The MIREC Study
Statut de l'article : révision en cours du manuscrit à Santé Canada. Il sera soumis au journal Environmental Health Perspectives.

6. Distinctions. Cuba

a. 2000: Meilleure étudiante étrangère dans le domaine académique
b. 2000: Meilleure étudiante étrangère dans le domaine de la recherche

7. Connaissances linguistiques

a. Internationales: niveau avancé en français et en espagnol, niveau intermédiaire en anglais.
b. Nationales: pkelle, soussou et malinké.

8. Bourses

a. Bourse MIREC Study 2016-2018. Québec. Canada
b. Bourse QTNPR 2012-2016. Québec. Canada
c. Septembre 2012 à Août 2014: Bourse d'exemption des frais supplémentaires de scolarité de la Faculté des Études Supérieures et Post-doctorales. Québec. Canada
d. Septembre 2011 à Mars 2012: Bourse de maîtrise de la Fondation du CHU Ste-Justine et Fondation des Etoiles (déclinée). Québec. Canada
e. Septembre 2011 à Août 2012: Bourse d'exemption des frais supplémentaires de scolarité. Accord Guinée-Canada
f. Septembre 2004 à Novembre 2008: Bourse de spécialité en obstétrique et gynécologie du Ministère de l'éducation de Cuba
g. Septembre 1997 à Juillet 2004: Bourse d'études médicales. Accord Cuba-Guinée

9. Expérience professionnelle

a. Décembre 2009 à Août 2011: engagement dans la fonction publique guinéenne comme professeure. Conakry. Guinée.
b. Février 2010 à Janvier 2011: Médecin au CHU d'Ignace Deen. Conakry. Guinée.
c. Avril 2010 à Juin 2010: Médecin à la clinique 'Ambroise Paré'. Conakry. Guinée.
d. Septembre 2004 à Novembre 2008: Médecin résidente à l'Hôpital Provincial Universitaire Gynéco-obstétrique de Santa Clara. Villa Clara. Cuba.

Annexe 8 : Les pièces jointes de l’annexe 4

Paediatric and Perinatal Epidemiology
Affiliated to the Society for Pediatric and Perinatal Epidemiologic Research

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Study design article

Cohort Profile: The Maternal-Infant Research on Environmental Chemicals Research Platform

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Abstract

Background: The Maternal-Infant Research on Environmental Chemicals (MIREC) Study was established to obtain Canadian biomonitoring data for pregnant women and their infants, and to examine potential adverse health effects of prenatal exposure to priority environmental chemicals on pregnancy and infant health.
Methods: Women were recruited during the first trimester from 10 sites across Canada and were followed through delivery. Questionnaires were administered during pregnancy and post-delivery to collect information on demographics, occupation, life style, medical history, environmental exposures and diet. Information on the pregnancy and the infant was abstracted from medical charts. Maternal blood, urine, hair and breast milk, as well as cord blood and infant meconium, were collected and analysed for an extensive list of environmental biomarkers and nutrients. Additional biospecimens were stored in the study's Biobank. The MIREC Research Platform encompasses the main cohort study, the Biobank and follow-up studies.
Results: Of the 8716 women approached at early prenatal clinics, 5108 were eligible and 2001 agreed to participate (39%). MIREC participants tended to smoke less (5.9% vs. 10.5%), be older (mean 32.2 vs. 29.4 years) and have a higher education (62.3% vs. 35.1% with a university degree) than women giving birth in Canada.
Conclusions: The MIREC Study, while smaller in number of participants than several of the international cohort studies, has one of the most comprehensive datasets on prenatal exposure to multiple environmental chemicals. The biomonitoring data and biological specimen bank will make this research platform a significant resource for examining potential adverse health effects of prenatal exposure to environmental chemicals.

Keywords: *biomonitoring, biological markers, environmental chemicals, pregnancy cohort study.*

Background

It is well recognised that *in utero* and early life exposures to elevated levels of some environmental chemicals can impact fetal and child health, and potentially chronic conditions of adulthood.[1,2] The global burden of disease attributable to selected chemicals amounts to at least 86 million disability-adjusted life years, with 54% of this burden borne by children under the age of 15 years.[3] The contribution of lead, methylmercury and organophosphate pesticides to IQ decrements in children under 6 years of age is substantial and exceeds those of many nonchemical risk factors.[4] It is generally recognised that prospective pregnancy or birth cohort studies incorporating exposure biomarkers during sensitive windows are required to examine potential health effects of developmental exposure to chemicals.[1]

In 2006, the government of Canada launched the Chemicals Management Plan (CMP), which set priorities for the assessment and management of hundreds of chemicals (http://www.chemicalsubstanceschimiques.gc.ca/plan/index-eng.php). A key element of the CMP is human biomonitoring, with the Maternal-Infant Research on Environmental Chemicals (MIREC) Study being one of the national initiatives.

Consultations were held with the US National Children's Study leaders on the possibility of developing a Canadian pregnancy cohort study in collaboration with the American initiative. However, it soon became apparent that a large Canadian cohort study modelled on the National Children's Study was unlikely. Thus, funding was secured to establish a new cohort building on the clinical infrastructures already established for a multi-site clinical trial.[5] The MIREC Study

(http://www.mirec-canada.ca; http://www.hc-sc.gc.ca/ewh-semt/contaminants/mirec/index-eng.php) is an interdisciplinary collaboration between Health Canada scientists and clinical and academic researchers, and was funded by Health Canada, the Ontario Ministry of the Environment, and a grant from the Canadian Institutes of Health Research.

This paper describes the objectives, study population and the data collected in the MIREC Study, and introduces the MIREC Research Platform, which also includes the MIREC Biobank and follow-up of some of the infants and children. While this research will serve as a resource for the original investigators, it is an example where the exposome is being used to discover new leads in reproductive and perinatal epidemiology.

Aims and objectives

A major aim of the MIREC Research Platform is to study the potential role of environmental chemicals on the health of pregnant women and their children. The primary objectives of the MIREC Study are the following: (i) to determine whether current non-occupational exposure to heavy metals, such as lead (as measured in maternal and infant biospecimens), is related to elevated maternal blood pressure or fetal growth restriction and (ii) to obtain national-level contemporary biomarkers of *in utero* and lactational exposure to priority environmental chemicals (e.g. heavy metals, phthalates, brominated flame retardants, bisphenol A [BPA]). Additional objectives include the following: (i) to obtain Canadian biomonitoring and survey data on smoking behaviour and exposure to tobacco smoke (active and passive) in pregnancy; (ii) to measure selected known or perceived beneficial substances in human milk, such as nutrients and minerals (e.g. vitamins D and E, complete fatty acids profile, folate, calcium and magnesium), relevant immunoprotective end points (e.g. secretory IgA, prolactin, lysozyme) and antioxidative enzymes in mature human milk; (iii) to undertake a comprehensive risk to benefits analysis for human milk (e.g. Schütte and colleagues[6]); (iv) to elucidate the oxidative stress pathways by analysing metabolite and proteomic biomarkers, and examining their association with heavy metal concentrations in maternal plasma, as well as associations with vasoregulatory components such as the plasma endothelins and free nitrite levels; (v) to explore candidate genetic polymorphisms that may explain differences in susceptibility to metals toxicity; and (vi) to examine vitamin D status during pregnancy.

Subsequent funding was obtained for several follow-up studies that form part of the MIREC Research Platform. A Biobank has been established to store biospecimens and data for future research on the health of pregnant women and their children. A process has been set up for investigators to request permission to access the MIREC Biobank (see http://www.mirec-canada.ca); however, privacy rules will not permit individual-level data to leave Canada.

Infrastructure and study design

Study population

The study sites were selected in part if they had established clinical obstetrical research infrastructure in place and also to represent different geographical regions of the country (Figure 1). Clinical site investigators from the 10 cities across Canada were asked to recruit a total of 2000 pregnant women from the general population who were attending prenatal clinics (ultrasound, midwife and/or doctor's clinics) during the first trimester of pregnancy (6 to <14 weeks). Recruitment took place between 2008 and 2011. The selected cities range from some of the largest in Canada, Toronto and Vancouver, where only 53% of the population identify English as their only

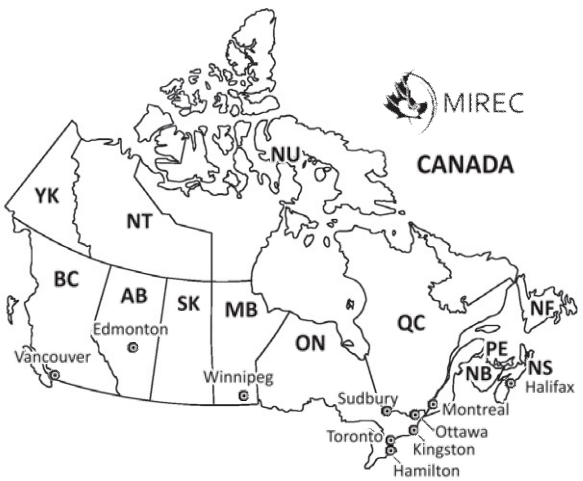

Figure 1. Map of Canadian Maternal-Infant Research on Environmental Chemicals (MIREC) sites.

mother tongue, to a smaller northern city such as Sudbury where 66% are English, and to Montreal where 52% identify their first language as French.

Eligibility criteria included ability to consent and to communicate in English or French, age 18 years or older, <14 weeks gestation, willing to provide a sample of cord blood and planning on delivering at a local hospital. Women with the following medical history were excluded from the study:

1 women who had known fetal abnormalities (e.g. hydatidiform mole), or known fetal chromosomal or major malformations in the current pregnancy; and
2 women who had a history of medical complications, including the following:
   - renal disease with altered renal function;
   - epilepsy;
   - any collagen disease, such as lupus erythematosus and scleroderma;
   - active and chronic liver disease (hepatitis);
   - heart disease;
   - serious pulmonary disease;
   - cancer;
   - haematological disorder (patient with anaemia or thombophilias will be included);
   - threatened spontaneous abortion (women with previous bleeding in the first trimester were included if the site documented a viable fetus at the time of recruitment); and
   - illicit drug use.

Data and biospecimen collection

Contacts were made with participants during each trimester, at delivery and in the early postnatal period (up to 10 weeks) to collect data and biospecimens (i.e. blood, urine, cord blood, meconium, breast milk and hair) for planned laboratory analyses (Figure 2). Under a separate consent form, additional biospecimens were collected to be stored in the MIREC Data and Biological Specimens Bank (MIREC Biobank). Questionnaires were administered by trained research staff to participants during the first and third trimesters to collect demographic, life style (e.g. smoking and alcohol), medical history, use of natural health products and medications, and potential sources of exposure data. Where possible, questions used in previous surveys and studies were used for comparability of results. A validated food frequency questionnaire was administered in the second trimester, along with blood and spot urine collection, blood pressure, clinical laboratory tests, and anthropometric measurements. Medical chart information was extracted at the first trimester ultrasound, second trimester and post-delivery time points. Blood pressure, height, weight and a proteinuria test were taken at each trimester.

Figure 2 provides details on participant contacts and biospecimen collection and analyses for environmental chemicals, nutrients, genetic factors, markers of oxidative stress and immunoprotective factors. Maternal urine was collected in Nalgene® containers (Thermo-Fisher Scientific Inc., Rochester NY, USA), and a Mère Hélène® bioliner (Mère Hélène, Quebec, Canada) was used on the diapers to collect meconium. All collection containers were pretested for phthalates and BPA, and field blanks were incorporated for each major chemical analyte. Post delivery, research staff visited the home of participants to collect samples of maternal milk and hair, and to complete a questionnaire on dietary factors. Women were asked to hand-express hind- and fore-milk over multiple days between 2 and 10 weeks post delivery; if they were having difficulty hand-expressing, a Medela® (Medela International, Zug, Switzerland) manual breast pump was provided, along with instructions for either manually expressing or using the breast pump to collect the milk. The milk was collected in 16-oz wide mouth amber I-CHEM® glass jars with fluoropolymer resin-liner polypropylene closure (Thermo Fisher Scientific, Rockwood, TN, USA) and 16-oz wide mouth TraceClean® clear plastic polyethylene jars (VWR International, Radnor, PA, USA). The breast pump was tested for possible contamination with phthalates and BPA.

Chemical analyses of maternal blood and urine, cord blood, and meconium were carried out by the Toxicology Laboratory, located in the *Institut national de santé publique du Québec* (https://www.inspq.qc.ca/ctq/Default.asp?Page=1&Lg=en), which is accredited by the Standards Council of Canada under ISO 17025 and CAN-P-43. The accuracy and precision of the analyses are evaluated on a regular basis through the laboratory's participation in external quality assessment programmes. DNA was extracted from maternal blood, and genotyping was performed by the *Plateforme de séquençage et de génotypage des génomes* at the *Centre de recherche du CHUL/CHUQ* (http://www.sequences.crchul.ulaval.ca/eng/index.html).

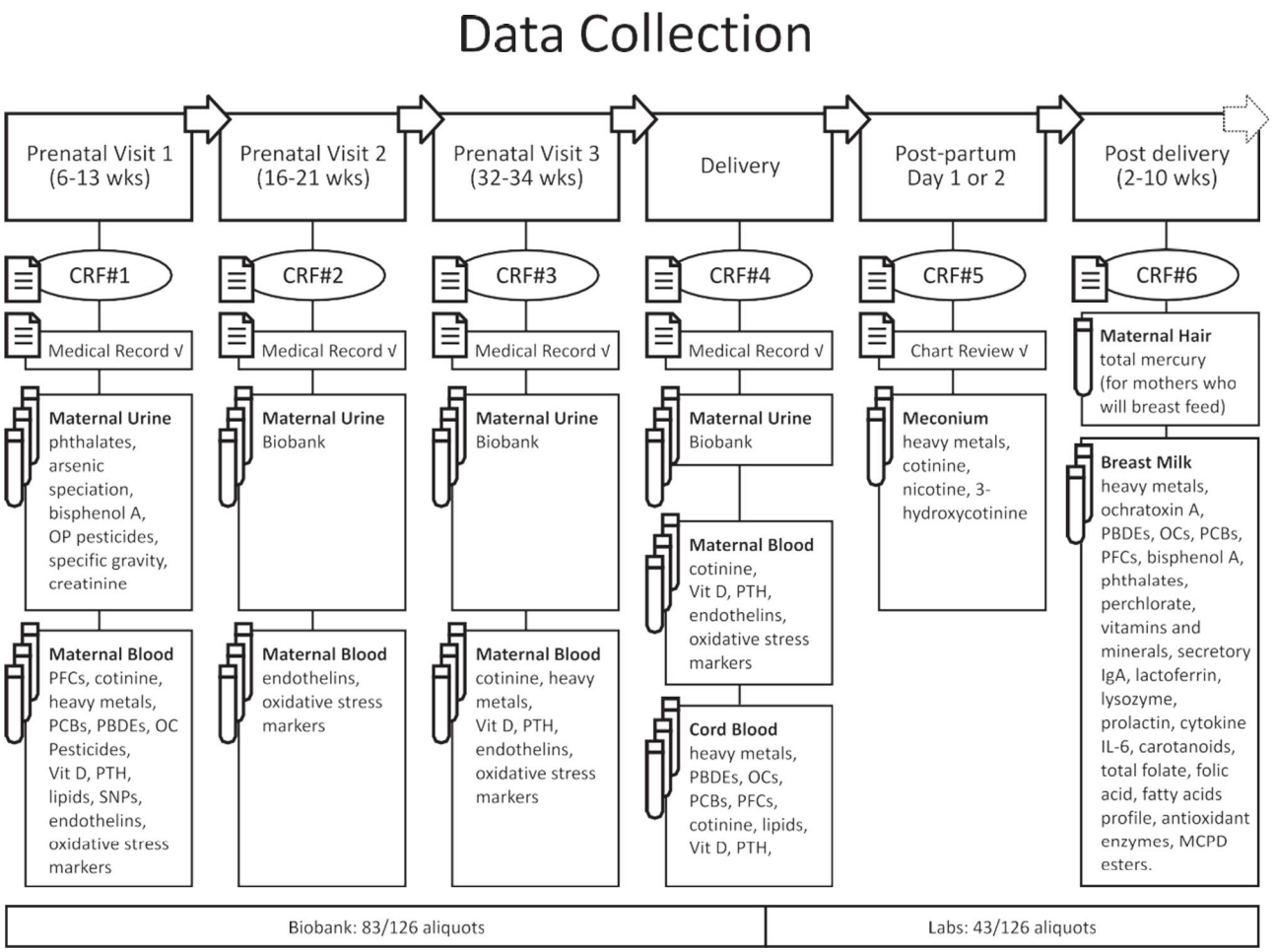

Figure 2. Data and biospecimen collection and analytes to be measured in the Maternal-Infant Research on Environmental Chemicals Study. CRF, case report form (questionnaire); OP, organophosphate pesticides; wks, weeks gestation; PCBs, polychlorinated biphenyls; PBDEs, polybrominated diphenyl ethers; PFCs, perfluorinated compounds; OC, organochlorines; Vit D, vitamin D; PTH, parathyroid hormone; SNPs, single-nucleotide polymorphisms; MCPD, 3-monochloropropane-1,2-diol.

Nutritional analyses of the maternal and cord blood samples, as well as the chemical and nutritional analyses of the breast milk, are being carried out by the laboratories of the Food Directorate at Health Canada. Markers of oxidative stress, inflammation and vasoregulation are being measured by scientists from the Environmental and Radiation Health Science Directorate of Health Canada.

All questionnaires and biospecimens were labelled with a unique ID and barcode, respectively. Data from questionnaires were entered into a database using single data entry with queries and verification of responses dealt with as needed (e.g. values outside range, internal inconsistencies). A separate database which tracked the collection and laboratory analysis of the biospecimens was also created. All blood, urine and breast milk were aliquoted into smaller cryovials and stored at -20 or -80°C as required.

Follow-up studies

In subsequent and ongoing follow-up studies, assessments of child health and development are being made on a subset of the cohort at various ages (see Figure 3). In the MIREC-ID Study (MIREC: infant development to 6 months), in-clinic assessments were performed on approximately 400 infants at birth and 6 months of age to measure growth, sensory function, behaviour and potential indicators of reproductive effects (e.g. anogenital distance). MIREC-CD3 (MIREC: child development at age 3), currently underway, is an online parental survey for children at 36

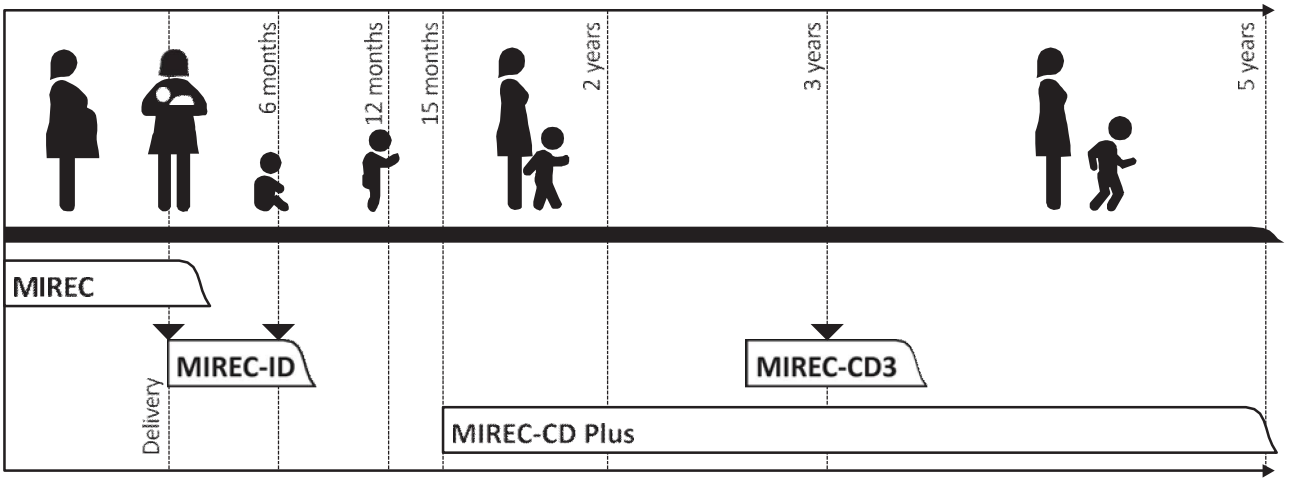

Figure 3. Maternal-Infant Research on Environmental Chemicals follow-up studies timelines.

months of age that is designed to examine potential effects of prenatal exposure to phthalates, BPA and organophosphate pesticides on child neurobehaviour [e.g. subscales of the Behavior Rating Inventory of Executive Function (BRIEF-P)]. The MIREC-CD Plus Study, currently in the planning stage, entails a home visit for children 15 months to 5 years of age to collect a sample of the child's blood and urine, to measure physical growth and only in children older than 2.5 years, to measure important determinants of child neurodevelopment (behaviour, general cognitive abilities, specific executive functions, and language and communications skills). In the MIREC-CD Plus Study, biological specimens will be collected to measure heavy metal exposure for those children under 3 years of age, with additional biospecimens placed in the Biobank. Delays in obtaining funding (which meant some children were too old for some of the assessments), insufficient funding and difficulties in obtaining ethics approvals at some of the recruitment sites have adversely affected our ability to include all of the cohort children in the follow-up studies. In the MIREC-CD Plus Study, to improve efficiency and reduce costs, recruitment is being restricted to six sites with the most MIREC children.

Participation in the MIREC Study

A total of 8716 women were approached at early (<14 weeks) prenatal clinics, 5108 were eligible and 2001 agreed to participate (39%) (Figure 4). Ethical considerations did not permit the collection of information on those who refused to participate, and no sampling frame was available to estimate the percentage of eligible women who were not approached. Among those who were ineligible to participate in the MIREC, 52% were not planning on delivering in the participating hospitals, 20% were outside the required gestational age at recruitment, and 14% were not willing to provide a sample of cord blood for the study. The ability to recruit eligible women varied considerably between cities, with a mean of 50% and ranging from a low of 13% in one city to over 90% in another, with no clear explanation for the differences.

Table 1 shows the participation rates by parity and year of recruitment. The mean and median gestational age at recruitment were 11.99 [standard deviation (SD) 1.51] and 12.43 weeks, respectively, with a minimum of 6.14 weeks and the 90th percentile of 13.57 weeks. After beginning participation in the MIREC Study, 18 women withdrew and asked that all their data and biospecimens be destroyed (0.9%), leaving 1983 participants. Additional loss to follow-up was due to withdrawals from the study (N = 48), fetal demise (N = 41), therapeutic abortion (N = 13) and mobility of the participants outside the study site (N = 14). The questionnaires were well responded to with the highest refusal or 'don't know' responses for family income (2.5% and 2.2%, respectively) and pre-pregnancy body mass index (BMI) (7.6% missing) (data not shown). Only 48 women (2.4%) did not consent to having their biospecimens and data stored in the Biobank.

The mean age of participants was 32 years, and 44% had not had a previous viable pregnancy (Table 2).

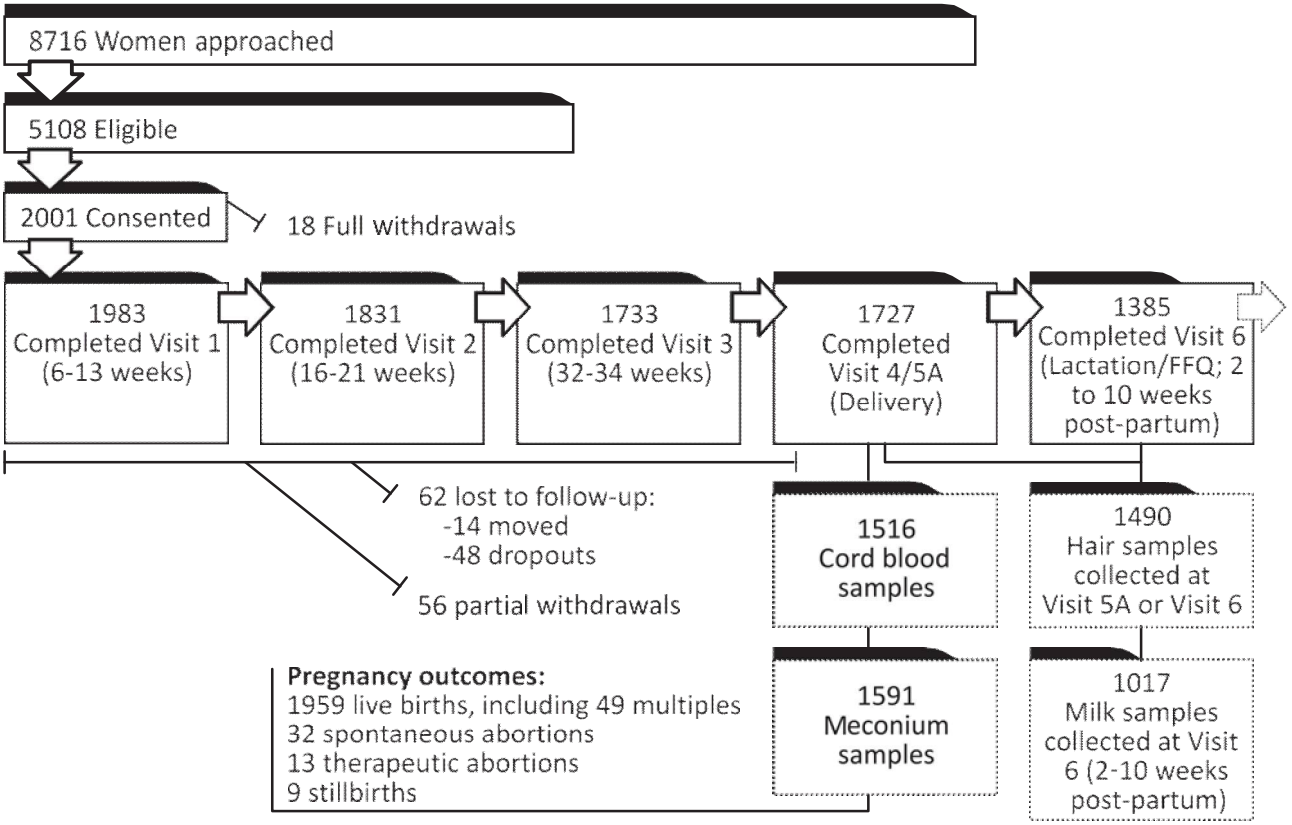

Figure 4. Recruitment and participation in the Maternal-Infant Research on Environmental Chemicals (MIREC) Study. FFQ: food frequency questionnaire.

Over 36% of the women were overweight or obese, based on their pre-pregnancy BMI.

Power calculations

The final sample for MIREC included information on 1983 mothers and 1959 livebirths. The dichotomous outcomes of low birthweight occurred in 5.7% of births, and preterm delivery occurred in 8.7% of births. With such incidence, the power is 86.3% to detect a ratio of geometric mean lead levels of 1.15 mg/dL in low vs. normal birthweight newborns. The power is 96.2% to detect a ratio of geometric mean lead levels of 1.15 mg/dL in preterm vs. term

Table 1. Maternal-Infant Research on Environmental Chemicals recruitment by calendar year and parity

	Parity									
	0 1		2		3 or more				Total	
Year of recruitment	%	*N*	%	*N*	%	*N*	%	*N*	%	*N*
2008	3.0	59	3.3	66	0.9	18	0.4	9	7.7	152
2009	17.4	345	15.6	309	4.4	87	1.3	26	38.7	767
2010	21.8	432	19.5	386	5.6	110	1.9	37	48.7	965
2011	1.9	38	2.0	39	0.8	16	0.2	4	4.9	97
Total	44.1	874	40.4	800	11.7	231	3.8	76	100	1981[a]

Definition of parity: number of previous viable pregnancies, not including index pregnancy.
[a]*N* = 2 missing information on parity.

Table 2. Comparison of Maternal-Infant Research on Environmental Chemicals (MIREC) participants with data on Canadian births (2009) and female participants in the biomonitoring component of the Canadian Health Measures Survey (CHMS) Cycle 1, (2007–09)[14,15]

	MIREC participants (2008–11)	Canadian births, 2009	CHMS (2007–09) women 20–39 years of age[a] [95% confidence interval]
Parity (%): Number of previous viable pregnancies			
0	44.1%	44.0%	49.7% [41.5, 57.9]
1	40.4%	35.0%	16.7% [12.8, 20.6]
2	11.7%	13.6%	25.0% [17.2, 32.7]
3+	3.8%	7.4%	8.6% [5.2, 12.1][b]
Maternal age (%) (years)			
<20[c]	0.7%	4.1%	
20–24	6.4%	15.2%	
25–29	23.2%	30.7%	
30–34	35.8%	31.7%	
35+	34.1%	18.3%	
Mean age (years) (SD)	32.2 (5.10)	29.4	29.9 [29.0, 30.8]
Gestational age (weeks)			
Mean (SD)	39.2 (1.98)		
Median	39.4		
Preterm birth (%): <37 weeks			
Yes	8.7%	7.7%	
Birthweight (g)			
Mean (SD)	3402 (581.89)	3364	
Median	3420	3391	
Low birthweight (<2500 g) (%)			
Yes	5.7%	6.1%	
Mother born in Canada (%)			
Yes	81.3%	72.7%	77.8% [67.6, 88.1]
Infant gender (%)			
Male	52.6%	51.3%	
Female	47.4%	48.7%	
Maternal education (%)		[d]	Highest level of education in household
High school or less	8.8%	26.8%	10.7% [4.6, 16.8][b]
Some college	5.3%		7.9% [3.3, 12.6)][b]
College diploma	23.6%	37.0%	38.9% [30.0, 47.9]
University degree	62.3%	35.1%	42.4% [28.4, 56.5]
Marital status of mother (%)			
Married or common law	95.3%	60.1%	58.7% [51.4, 66.1]
Divorced	0.2%	0.9%	1.4% [0.7, 2.1)][b]
Separated	0.2%	0.4%	2.1% [0.6, 3.5][b]
Single	4.2%	27.2%	37.8% [30.5, 45.0]
Other/Unknown	0.05%	11.5%	[e]
Multiple births (%)			
Yes	2.5%	3.3%	
Fetal death (Š20 weeks) (% of total births)			
Yes	0.5%	0.7%	
Smoking status at first visit (<14 weeks) (%)		During last 3 months of pregnancy[d]:	
Never	61.7%	89.5% did not smoke	59.9% [52.7, 67.0]
Former	26.3%		19.3% [15.4, 23.1]
Quit during pregnancy	6.1%		
Current smoker	5.9%	10.5%	20.8% [16.2, 25.5]
Body mass index (kg/m^2)			
Underweight (<18.50)	2.9%		5.3% [2.1, 8.6][b]
Normal (18.50–24.99)	60.5%		50.4% [39.8, 60.9]
Overweight (25.00–29.99)	22.0%		23.3% [16.2, 30.5]
Obese (>29.99)	14.6%		20.9% [15.6, 26.3]
Household income from all sources[f]			
<$20 000	3.9%		10.7% [5.4, 16.0][b]
$20 000–30 000	3.5%		5.0% [2.7, 7.3][b]
$30 001–40 000	4.9%		9.0% [4.0, 13.9][b]
$40 001–50 000	5.1%		9.3% [5.0, 13.6][b]
$50 001–60 000	5.1%		9.8% [6.6. 13.0]
$60 001–80 000	14.9%		18.2% [14.5, 21.8]
$80 001–100 000	19.6%		9.6% [4.3, 14.9][b]
>$100 000	38.2%		20.6% [16.1, 25.1]
No response	4.7%		7.8% [3.7, 12.0][b]

[a]Estimates weighted for the CHMS complex survey design.

[b]High sampling variability associated with these estimates. Results should be interpreted with caution.

[c]Lowest age category defined as 18 to <20 for MIREC and under 20 years for Canadian births. [d]Source: Reference 17.

[e]Estimates of unacceptable quality and suppressed by Statistics Canada.

[f]$1 difference in categories between MIREC and Statistics Canada.

deliveries. For the same population size, and birthweight as a continuous variable, the power is 91.3% to detect the risk factor, with an effect size of 0.075 SD.

Comment

Ethical considerations

The research protocol, questionnaires, consent forms, and recruitment posters and pamphlets were reviewed and approved by human studies research ethics committees, including the Research Ethics Board at Health Canada and the research ethics committee at the coordinating centre at Ste-Justine's Hospital in Montreal, as well as >10 academic and hospital ethics committees across Canada – a very time-consuming process. Any changes made to the protocol or survey instruments (amendments) were approved by all ethics committees. Separate consent forms were developed for the data and biospecimen repository (Biobank) and for the infant follow-up studies. Participants could partially (data and biospecimens retained) or completely (all data and biospecimens destroyed) withdraw from the study. Ethical considerations did not permit the collection of any information on the non-participants to examine possible selection bias.

The approach to reporting biomonitoring results to participants was a major point of discussion between the study investigators and the hospital ethics committees at the coordinating centre and recruitment sites.[7] Our initial proposal to the ethics committees was that individual biomonitoring results would be provided to participants (if they so requested) at the conclusion of the data collection and the analysis phase of the initial cohort study, along with information on potential sources of exposure. The exception would be chemicals for which there were health-based tissue guidelines [i.e. blood lead, mercury and cadmium (in the case of cadmium, the workplace health guideline was used)]. Any participant with blood levels exceeding the guidelines would be informed as soon as possible and provided with information on reducing exposure, including possible follow-up blood measurements. However, the hospital ethics committees required that only biomonitoring results exceeding health-based tissue guidelines where preventive or treatment options were available could be shared with participants through their health care provider. This latter process was, therefore, used to share maternal blood concentrations of lead, mercury and cadmium that exceeded established guidelines with the participant's physician. The physicians were also provided with guidance documents to help them identify potential sources of exposure for their patients and to help them interpret the individual results. A physician with expertise in environmental epidemiology of these metals was made available to the participant's physicians to provide further advice, as needed. In the province of Quebec, a number of chemical substances, including lead, mercury, cadmium and manganese, as well as the dialkyl phosphate metabolites, fall under reportable diseases legislation [maladies et intoxications à déclaration obligatoire (MADO)]. Therefore, our Montreal sites were legally obliged to report elevated results to the public health department, according to the MADO guidelines. Recently, we have had a request from an MIREC participant for her individual biomonitoring results for environmental chemicals, and we have been given permission by the ethics committees to provide these results to her with the disclaimer that we cannot provide any interpretation of her results as there is currently no scientific knowledge available to interpret these results at the individual level.

Perspectives

MIREC is a multisite study with a population of obstetric patients from across Canada. One of the unique features of this study is the most extensive assessment of prenatal and lactational exposure to environmental chemicals at multiple time points, but especially in early pregnancy when the fetus is likely most sensitive to toxic chemicals. In addition, the establishment of the Biobank will facilitate future research on additional chemicals and genomic and nutritional susceptibility factors. As such, MIREC is directly responsive to the exposome paradigm,[8] measuring endogenous factors such as oxidative stress, specific external exposures such as chemicals and diet, and general external exposures including economic and educational factors. By doing so, the findings from the MIREC Research Platform will contribute to the identification and quantification of exposomes during sensitive windows of reproduction and development, as recently recommended by Buck Louis and colleagues.[9]

The results of MIREC may not be generalisable to the Canadian population or to each of the recruitment

sites as the study is not population-based. Past experience has highlighted the difficulties in trying to assemble a population-based pregnancy/birth cohort that may be less representative as the cohort is followed over time.[10–12]

The MIREC participation rate of 39% is consistent with participation rates of several large prospective cohort studies.[13] Similar to other pregnancy cohort studies (e.g. Bornehag *et al.*[11]), participants in MIREC tended to be older, more educated, born in Canada, married and less likely to be a current smoker than the Canadian population giving birth in 2009 (Table 2).[14] The MIREC participants were, however, more similar to a population of women 20–39 years of age participating in the biomonitoring component of the population-based Canadian Health Measures Survey (Cycle 1 2007–09).[15,16]

In summary, the multiple contacts with study participants starting in the first trimester, and the collection of data and biospecimen analysis for multiple key chemical risk factors in the MIREC Study, should provide important information on several hypotheses related to prenatal exposure to environmental chemicals and potential adverse health effects for the pregnant woman and her child. The major challenges are funding to maintain the Biobank and to follow the cohort as the child ages, as well as keeping the families actively participating in the various ancillary studies, while limiting biases that might affect the internal validity of the study.

Acknowledgements

The cooperation of MIREC participants in providing data and biospecimens is gratefully acknowledged, as is that of the staff at each recruitment site who diligently recruited and followed the participants throughout their pregnancy. We would like to thank Susanne Andersen, Annick Tremblay, Gabriel Abad, Rosario Maticorena, Lucia Arango, Gloria Sanchez, Fabiola Botello and Trinidad Madrid from the coordinating centre at CHU Sainte-Justine for carrying out the day-to-day work of the study. Chantal Roy is thanked for her crucial role at the coordination centre during the early stages of the study. The authors are especially grateful to Dr. Sheryl Bartlett and Douglas Haines at Health Canada for their endless support for this project, and Dr. Lesbia Smith and Dr. Donald Cole for their critical role in guidance and support of the interpretation of the maternal blood heavy metal results. The MIREC Research Platform is supported by the Chemicals Management Plan of Health Canada, the Canadian Institutes for Health Research (grant # MOP – 81285) and the Ontario Ministry of the Environment.

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Maternal-Infant Research on Environmental Chemicals (MIREC): A National Profile of In Utero and Lactational Exposure to Environmental Contaminants

MIREC
Maternal-Infant Research on Environmental Chemicals

Project Description

Investigators:
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Funding agencies and research partners:
Health Canada – Safe Environments Programme, Tobacco Control Programme, Product Safety Programme, Food Directorate
Ontario Ministry of the Environment
Canadian Institutes of Health Research

Duration:
5 Years, December, 2006 – March, 2012 (or as soon as the Project Gets Approved)

Proposed Budget: $10.7 M over 5 years (based on current funding commitments)
Ste. Justine's Hospital: $7.2 M over 5 years
- Study coordination; patient recruitment; data and biospecimen collection, processing and shipping; data management; biospecimen storage; communication of results to patients/physicians

Food Directorate: In-kind contribution over 5 years (approximate value $3 million)
- Human milk survey: laboratory of human milk for priority environmental chemicals, relevant nutrients and immunoprotective constituents; advice on interpretation of results

CTQ, INSPQ: $3.5 M over 5 years
- Laboratory analysis of biospecimens for priority environmental contaminants, advice on interpretation of results

This Project Description Includes:
The Research Proposal
The Proposed Budget
The Study Questionnaires (Case Report Forms)

The Sample Informed Consent Forms
Instructions for Shipping and Handling Biological Specimens
Recruitment Poster and Pamphlet

Maternal-Infant Research on Environmental Chemicals (MIREC): A National Profile of In Utero and Lactational Exposure to Environmental Contaminants

Summary:

The measurement of levels of environmental contaminants in blood, urine and human milk (i.e. biomonitoring) is an important tool to assess human exposures and health risk. While it is expected that Statistics Canada's Canadian Health Measures Survey (2007-09) will provide much needed national data on exposure of the Canadian population to several priority environmental contaminants, it will not collect data for two of the most susceptible and vulnerable populations - the pregnant woman and her fetus. These two populations are very difficult to sample in a national population-based survey because of their relative rarity, particularly for specific sensitive periods during the pregnancy. Sampling this kind of population - a pregnancy cohort- requires specialized techniques. There was an initiative that focused on these two vulnerable populations - the International Trial of Antioxidants for the Prevention of Preeclampsia (INTAPP Study), funded by CIHR and led by Dr. William Fraser at Ste. Justine's Hospital in Montreal. Through the INTAPP study, a network of 13 clinical sites across Canada and the infrastructure to support a multi-center study were established. It is proposed that we build on this valuable resource to establish a national-scale pregnancy cohort study which would meet the needs of the financial supporters and the investigators. Health Canada's Healthy Environments and Consumer Safety Branch has identified a need for data on maternal exposure to and health effects of specific priority environmental contaminants and is prepared to contribute to the costs of collecting this data. The Ontario Ministry of the Environment has already provided some funds for this work. The Health Products and Food Branch has in parallel identified a need for data on nutritional, environmental chemicals and immunoprotective constituents in human milk and are prepared to cover the cost of following the cohort to obtain human milk. An application to CIHR has been successful in contributing funds to the project to study the role of heavy metals and nutrition in adverse pregnancy outcomes.

While the neurotoxicity of lead and mercury in children is well known, the effects of low prenatal body burdens of lead and other heavy metals such as cadmium, arsenic, manganese and mercury on maternal and infant health are less understood. Preliminary evidence suggests that maternal blood lead levels below the current CDC action limit of 10 µg/dl have adverse effects on blood pressure, fetal growth and later child intellectual development, resulting in possible permanent effects. Little is known about how to reduce exposure to these low levels. Experimental animal and cross-sectional epidemiologic studies are also suggesting that nutritional factors such as antioxidant vitamin intake (Vitamins C and E) and calcium and other essential elements mitigate heavy metal toxicity.

Objectives:

The primary objective of this study is to determine whether contemporary non-occupational-level heavy metal exposure as measured by maternal and fetal body burdens is related to elevated blood pressure, gestational hypertension and fetal growth retardation among women and their infants. A second primary objective is to establish national-level contemporary measurements of priority environmental chemicals in pregnant women and their newborn infants and to measure environmental chemicals and food processing induced chemicals (selected persistent organic pollutants (POPs), heavy metals, perchlorate, mycotoxins, bisphenol A, 3-chloropropane-1,2-diol (3-MCPD) and 2-chloropropane-1,3-diol (2-MCPD) esters), selected nutrients (priority vitamins, complete fatty acids profile (including trans and omega-3 fatty acids), suite of minerals) and relevant immunoprotective endpoints in mature human milk.

Methods:

A cohort of 2,000 women will be recruited during the first trimester of pregnancy over a 2 – 3 year enrolment period at 8-10 sites across Canada. Contacts during each trimester of pregnancy will be made with each woman during regularly scheduled clinic visits to collect questionnaire data, medical history, and maternal blood and urine. Post-delivery, additional questionnaire data, medical data, cord blood, infant meconium, and breast milk and maternal hair will be collected. These biological specimens will be analyzed for heavy metals (lead, cadmium, arsenic, manganese and total mercury) and will represent maternal and fetal body burdens of these metals during different time windows (and periods of vulnerability) during the pregnancy. In addition, maternal blood and urine samples from at least a sub-sample of the women (depends on availability of funds) recruited to the study will be analyzed for brominated flame retardants (PBDEs) and other persistent organic pollutants (PCBs, OC pesticides), plasticizers (phthalate metabolites, and bisphenol A), organophosphate pesticides, cotinine and surfactants (perfluorinated chemicals [PFCs]). Biological specimens from the remaining members of the cohort will be stored and analyzed for these environmental chemicals when additional funds are secured. Human milk samples will be collected from a subset of the cohort for analysis of selected organic and inorganic contaminants (PBDEs, PCBs, OC pesticides, PFCs, perchlorate), heavy metals, mycotoxins, plasticizers, 3-chloropropane-1,2-diol (3-MCPD) and 2-chloropropane-1,3-diol (2-MCPD) esters, minerals and immunoprotective endpoints. At clinic visits during each trimester and at delivery, blood pressure measurements and infant birth weight and gestational age will be recorded according to a strict protocol. With these prospective data, we will be able to measure the potential health effects of heavy metal body burdens on the mother (elevated blood pressure) or fetus (fetal growth). We will also explore mechanistic pathways for toxicity by measuring various oxidative stress markers and gene polymorphisms associated with heavy metal metabolism or toxicity.

Significance:

This research will provide new knowledge on maternal and fetal toxicity of heavy metals in a population of Canadian women. Identification of potential protective factors such as antioxidant vitamin supplements and calcium intake will be of public health significance if it translates into reducing the potential immediate and longer term sequelae of these environmental hazards. As well, for the first time in Canada, national-level biomonitoring data for pregnant women will be available for use in risk assessment and risk management of environmental chemicals. This study will provide invaluable insight into smoking behaviour, use of cessation therapies, which are being encouraged for use during pregnancy, and smoking restrictions in the home. This information will be useful for Governments and public health practitioners in developing policies and programs to encourage pregnant women to quit smoking and to avoid exposure to Environmental Tobacco Smoke (ETS). For the human milk component, the national scope of the study will fulfill an important knowledge gap for nutrients, immunoprotective constituents and environmental chemicals, as existing published data are either non-existent, are limited in numbers of subjects/geographic range and/or are outdated. Given that human milk is the predominant and often the sole-source of food for infants, reference standards for definition of infant nutritional needs are based on levels in human milk and as such, this research will serve to re-examine Health Canada's existing policy regarding infant nutritional needs. In addition, concurrent measurements of nutrients and immunoprotective constituents, alongside environmental chemicals, in human milk will provide the unique opportunity to conduct a comprehensive risk: benefit analysis. Overall, the results of this research are expected to strengthen health risk assessments (including promotion of breastfeeding) and to support measures to reduce release of contaminants into the environment, and limit exposure of the general population.

Maternal-Infant Research on Environmental Chemicals (MIREC): A National Profile of In Utero and Lactational Exposure to Environmental Contaminants

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Maternal-Infant Research on Environmental Chemicals (MIREC): A National Profile of In Utero and Lactational Exposure to Environmental Contaminants

I - Background and Rationale

1. Prenatal Exposure to Heavy Metals

Heavy metals are a public health concern given their toxicity, especially in vulnerable fetuses, their long biological half-life in humans and their ubiquity in the environment. Sources of exposure include contaminated food, drinking water, air, dust, tobacco smoke, and dental amalgams. In Canada, there is no national biomonitoring program in place to measure exposure of the general population, pregnant or lactating women in particular to heavy metals. The only national survey which measured heavy metals in blood was conducted almost 3 decades ago (Health-and-Welfare-Canada). Maternal or umbilical cord blood heavy metal data are available for small select populations such as First Nation Cree (Hanning, Sandhu et al. 2003), the Inuit (Levesque, Duchesne et al. 2003), subsistence fishing groups (Belles-Isles, Ayotte et al. 2002), or from populations in Montreal (Smargiassi, Takser et al. 2002) and southern Quebec (Rhainds, Levallois et al. 1999; Lafond, Hamel et al. 2004; Takser, Lafond et al. 2004).

Although current exposures to lead in Canada are expected to be much lower than 25 years ago, lead toxicity may remain a problem due to its accumulation and persistence in bone. Over 95% of lead in a typical adult is stored in bone (Schroeder and Tipton 1968; Barry and Mossman 1970) with a half-life estimated in decades (Rabinowitz 1991). Women who were chronically exposed to lead throughout their childhood may reach reproductive age with a significant bone lead burden (Sanin, Gonzalez-Cossio et al. 2001). Recent studies suggest that during periods of increased bone resorption, lead stored in bones may become bioavailable. Skeletal lead stores can be mobilized into blood during pregnancy and lactation (Gulson, Jameson et al. 1997; Gulson, Mahaffey et al. 1998) with possible toxic implications to the mother and fetus, as well as during menopause when bone demineralization occurs (Silbergeld, Schwartz et al. 1988). Blood lead levels appear to decrease with parity, implying that the greatest risk of lead toxicity lies with first pregnancies (Manton, Angle et al. 2003). Lead freely crosses the placental barrier (Goyer 1990) and bone lead contributes a substantial fraction of the lead in cord blood (Gulson, Jameson et al. 1997). It has been estimated that approximately 79% of maternal skeletal lead is mobilized during pregnancy and transferred to the infant via cord blood (Gulson, Mizon et al. 2003). Maternal blood lead (Sanin, Gonzalez-Cossio et al. 2001) and mercury (Bjornberg, Vahter et al. 2003; Bjornberg, Vahter et al. 2005) are strong and significant predictors of infant blood levels at birth.

There is also evidence to suggest that a woman's exposure to lead changes during the course of pregnancy following a U-shape curve with significantly elevated levels during later pregnancy (Rothenberg, Karchmer et al. 1994; Hertz-Picciotto, Schramm et al. 2000; Tellez-Rojo, Hernandez-Avila et al. 2004). Little is known about the distribution of and consequent bioavailability of many of the other heavy metals during each trimester of pregnancy.

2. Reproductive Toxicity of Heavy Metals

Mercury can pass from mother to child *in utero* and can produce long-lasting harm to the child's neurological development (Grandjean, Murata et al. 2004). Lead has been widely studied for its neurobehavioural effects in children with subtle deficits occurring at blood lead levels below 5 μg/dl (Lanphear, Dietrich et al. 2000; Canfield, Henderson et al. 2003; Emory, Ansari et al. 2003) and to a lesser extent for its association with elevated blood pressure (Korrick, Hunter et al. 1999; Glenn,

Stewart et al. 2003; Nash, Magder et al. 2003; Telisman, Pizent et al. 2004) and impaired fertility in men (Pant, Upadhyay et al. 2003; Shiau, Wang et al. 2004) and women (Chang, Cheng et al. 2006) and delayed puberty in girls at levels of only 3 μg/dl (Selevan, Rice et al. 2003; Wu, Buck et al. 2003). In a national US survey, correlations between blood lead levels and serum follicle stimulating hormone and luteinizing hormone were also observed (Krieg Jr., 2007). *In utero* lead exposure has resulted in significantly poorer Mental Development Index scores at 12 and 24 months in infants (Hu, Tellez-Rojo et al. online 19 July 2006), reduced IQ in 6-10 year olds (mean 8 μg/dl) (Schnaas, Rothenberg et al. 2006) and elevated systolic blood pressure in children (Gump, Stewart et al. 2005). It has also been suggested that early exposure to lead may be a risk factor for childhood asthma and atopy (Dietert, Lee et al. 2004). An analysis of the dose-response for lead has concluded that the Centers for Disease Control and Prevention action limit of 10 μg/dl for children fails to protect against most damage and economic cost attributable to lead exposure (Rothenberg and Rothenberg 2005). Only recently have studies attempted to understand patterns and determinants of blood lead during pregnancy and its effects on maternal and infant health. The effect of historical lead exposures (endogenous sources based on a lifetime of exposure) on pregnancy and the newborn is still not well documented.

Although not conclusive, several studies have reported adverse effects of heavy metal exposure on fetal growth. Maternal lead exposure has been associated with premature rupture of membranes and preterm delivery (Angell and Lavery 1982; Falcon, Vinas et al. 2003), low birth weight and preterm birth (Andrews, Savitz et al. 1994) and intrauterine growth retardation (Srivastava, Mehrotra et al. 2001). Arsenic exposure may also play a role in increasing the risk of low birth weight (Hopenhayn, Ferreccio et al. 2003; Yang, Chang et al. 2003). Cord blood (Zhang, Zhao et al. 2004) and maternal blood (Nishijo, Tawara et al. 2004) cadmium levels may negatively affect neonatal birth height. Cadmium, unlike lead and mercury, does not easily cross the placental barrier (Osman, Akesson et al. 2000; Iyengar and Rapp 2001) but may affect placental function and consequently fetal growth and development (Yang, Julan et al. 2006).

Although lead and mercury have been extensively studied, research is limited on the human reproductive effects of low level exposure to other heavy metals. More recent studies indicate that adverse health effects are occurring at lower exposure levels than previously anticipated. There is also evidence to suggest that the toxicokinetics for heavy metals changes throughout the course of pregnancy (Hopenhayn, Huang et al. 2003) which might increase susceptibility to toxic effects during pregnancy.

3. Heavy Metals-Induced Effects on Hypertensive Disorders during Pregnancy

A number of adverse outcomes have been associated with hypertension during pregnancy including preterm birth, fetal growth retardation, perinatal death, acute renal or hepatic failure, antepartum haemorrhage, postpartum haemorrhage and maternal death (Collins and Wallenburg 1989; Brown, Hague et al. 2000; Report-of-the-National-High-Blood-Pressure-Education-Program-Working-Group-on-High-Blood-Pressure-in-Pregnancy 2000). Even a higher blood pressure within normotensive limits during the second half of pregnancy is associated with reduced birth weight (Churchill, Perry et al. 1997). Hypertension arising for the first time during pregnancy (from 20 weeks gestation) ranges from gestational hypertension through proteinuria and multi-organ dysfunction (preeclampsia) to seizures (eclampsia) (Roberts, Algert et al. 2005). In one of the few large population-based studies of maternal morbidities associated with hypertension in pregnancy, 9.8% of the women had a hypertensive disorder, including 0.6% with chronic hypertension, 4.2% with preeclampsia, and 4.3% with gestational hypertension (Roberts, Algert et al. 2005). Although one in 10 women giving birth suffers

from a hypertensive disorder in pregnancy, there is still a paucity of data on risk factors and their mechanism of action that can be used to prevent the sequelae in women and their infants.

In a large cross-sectional survey (NHANES III), no consistent relationship was found between blood pressure and blood lead levels in American adults (Den Hond, Nawrot et al. 2002). However, a very recent cross-sectional survey of older US adults reported that blood lead was a strong and consistent predictor of both systolic and diastolic blood pressure and suggested that lead has an acute effect on blood pressure via recent dose and a chronic effect on hypertension risk via cumulative dose as measured by bone lead (Martin, Glass et al. 2006). Furthermore, there is a small but accumulating body of evidence that suggests that exposure to heavy metals such as lead may play a role in the etiology of gestational hypertension, and preeclampsia. How and when exposure was measured may have affected the results. A small but statistically significant relationship between blood lead concentrations in the umbilical cord (Rabinowitz, Bellinger et al. 1987; Harville, Hertz-Picciotto et al. 2005) and in third trimester pregnant immigrant women (Rothenberg, Manalo et al. 1999) and maternal blood pressure has been reported. A more recent study of 667 Latino and African-American women found that calcaneus lead concentration, representing cumulative lifetime exposure to lead was significantly related to hypertension risk during pregnancy (Rothenberg, Kondrashov et al. 2002).

In a case-control study of gestational hypertension, cases (n=30) had significantly higher blood lead levels (mean 9.6 μg/dl) than normotensive controls and lead: ionized calcium ratio showed a stronger association with blood pressure than blood lead alone (Magri, Sammut et al. 2003). Another study of 705 women found that maternal blood lead concentrations (mean 1.2 μg/dl) were significantly related to hypertension in pregnancy/toxemia (Sowers, Jannausch et al. 2002). Concentrations of lead and manganese were significantly correlated with blood pressure in a study of pregnant women without occupational exposure to metals (Vigeh, Yokoyama et al. 2004).

Blood lead levels have also been shown to be elevated in women with preeclampsia in one study (Dawson, Evans et al. 2000) but not in two earlier studies (Angell and Lavery 1982; Rabinowitz, Bellinger et al. 1987). A more recent case-control study reported significantly increased risks of preeclampsia associated with umbilical cord blood levels of lead and manganese (Vigeh, Yokoyama et al. 2006). It is noteworthy that the mean cord blood lead levels in this study were 4.3 μg/dl for the cases, well below the public intervention level of 10.

Cadmium has been hypothesized to play a role in the etiology of eclampsia (Semczuk and Semczuk-Sikora 2001) and preeclampsia (Eisenmann and Miller 1995; Chisolm and Handorf 1996; Dawson, Evans et al. 1999). One study has reported that hypertension in pregnant women smokers is related to significantly higher blood cadmium concentrations (Kosanovic, Jokanovic et al. 2002). An adverse association between mercury exposure at background levels and systolic blood pressure has been observed among non-fish-consuming young and middle-aged women in the US (Vupputuri, Longnecker et al. 2005) which suggests that mercury may also impact on hypertension risk in pregnant women. Arsenic exposure may also be a contributing factor in the development of hypertension by impairing vasomotor tone (Lee, Jung et al. 2003).

4. Mechanisms of Heavy Metal-Induced Effects on Blood Pressure

Identifying and understanding the mechanism of action of any factors that can reduce the risk of elevated blood pressure, gestational hypertension or preeclampsia will improve maternal and infant health outcomes. The pathogenesis of lead-induced hypertension is likely multi-factorial including mechanisms such as (Vaziri and Sica 2004):

a) oxidative stress associated with inactivation of endogenous nitric oxide and down-regulation of soluble guanylate cyclase by reactive oxygen species (ROS), leading to functional deficiency in nitric oxide;
b) heightened sympathetic activity and plasma norepinephrine together with depressed vascular and elevated renal beta-adrenergic receptor density;
c) elevated plasma angiotensin-converting enzyme (ACE) activity, plasma renin activity (PRA), angiotensin II (Ang-II), and aldosterone levels;
d) increased kininase I and kininase II activities;
e) lead-induced inhibition of vascular smooth muscle Na (+)-K (+) ATPase, leading to a rise in cellular Na(+) and hence Ca(2+);
f) a possible rise in endothelin and thromboxane generation.

Endothelin is a potent vasoconstrictor which is produced in and is active in the uteroplacental vasculature and is down-regulated by vasodilators such as nitric oxide (Thaete, Dewey et al. 2004). Maternal endothelin 1 (ET-1) and fetal ET-1 concentrations are significantly higher in women with pregnancies complicated with intrauterine growth retardation (especially in women with preeclampsia) than those with normal pregnancies (Arslan, Yazici et al. 2004). Enhanced endothelin-1 production has also been shown in women with preeclampsia (Ajne, Wolff et al. 2003). In a recent case-control study, plasma concentrations of malondialdehyde (an indicator of lipoperoxidation), endothelin-1, fibronectin, and sE-selectin were significantly elevated whereas nitric oxide was significantly decreased in women with preeclampsia compared to healthy women (Aydin, Benian et al. 2004).

During pregnancy there is a high energy demand of many bodily functions and an increased oxygen requirement which produces more reactive oxygen species. Uncontrolled production of these reactive oxygen species results in oxidative stress and can result in significant damage to cell integrity, a major cause of maternal and fetal morbidity (Gitto, Reiter et al. 2002). Significant correlations have been reported between indicators of lead toxicity and oxidative stress in occupationally-exposed workers (Dursun, Dogan et al. 2001; Gurer-Orhan, Sabir et al. 2004). Oxidative stress may also play a role in the toxicity of other heavy metals such as nickel (Merzenich, Hartwig et al. 2001), cadmium (Xu, Maki et al. 2003), arsenic (Liu, Trimarchi et al. 2003), and mercury (Qian, Falahatpisheh et al. 2001).

Oxidative stress markers include indicators of protein oxidation (hydroxylation)/nitration, lipid oxidation, and DNA damage markers. Oxidative stress pathways involve reactive oxygen species (ROS)-mediated products and reactive nitrogen species (RNS)-driven products with a selection of one pathway or selected free radical species over the others under specific pollutant exposure and disease conditions (Many, Hubel et al. 2000; Okatani, Wakatsuki et al. 2000; Aydin, Benian et al. 2004; Kumarathasan, Blais et al. 2005). Depending on treatment conditions, either hydroxylation or nitration reactions can be predominant, and thus the markers relevant to this condition specific pathway are upregulated. These markers can be specific not only to exposure conditions but also are dependent on the biological region that is being examined. Only by selecting study markers relevant to each of these pathways can one have a clear understanding of the mechanisms. Also, oxidative stress pathways are implicated in the homeostasis of vaso-regulation, for instance, by interfering with endothelinergic mechanisms (Wedgwood, McMullan et al. 2001; Chen, Khan et al. 2004). In this study, clarification of such associations will allow us to understand the effects of any therapeutic intervention on improvements of pregnancy outcomes. The isoforms of mature peptides (ET-1, ET-2 and ET-3) and their precursors (BET-1, BET-2, BET-3) exhibit different extents of vasoregulatory properties. For example, of the three mature ETs, ET-1 is known to be the most potent vasoconstrictor. Endothelin-binding receptor expressions and binding kinetics can lead to either vasoconstriction or

vasorelaxation. Furthermore, the rate of conversion of precursor ETs to their mature peptides has been associated with disease pathologies (e.g. atherosclerosis) (Bohm, Johansson et al. 2002).

5. *Nutrient-Heavy Metals Interaction*

Nutritional status can play a role in altering absorption or susceptibility to toxicity of heavy metals. Bone demineralization may be caused by insufficient maternal dietary sources of calcium required for fetal bone growth (Hertz-Picciotto, Schramm et al. 2000) and result in increased levels of lead in the blood. A significant negative relationship between blood lead and calcium in third trimester women has been observed (Hertz-Picciotto, Schramm et al. 2000; Magri, Sammut et al. 2003). There is some evidence that calcium supplementation may be associated with a modest reduction in blood lead levels for lactating women (Hernandez-Avila, Gonzalez-Cossio et al. 2003; Ettinger, Tellez-Rojo et al. 2006). Calcium may act by decreasing lead absorption in the intestine or decreasing maternal bone resorption with subsequent release of lead (Johnson 2001).

Antioxidants such as vitamins C and E have been shown to mitigate hypertension or other toxicity in lead-exposed animals (Attri, Dhawan et al. 2003; Flora, Pande et al. 2003) and hence antioxidant therapy may play a role in the treatment of lead-induced hypertension and toxicity in humans (Hsu and Guo 2002; Vaziri 2002). In a large sample of US women of reproductive age, women in the lowest decile for serum ascorbic acid had significantly higher blood lead levels than those in the other deciles suggesting that women with poor nutritional status may be at greater risk for lead toxicity (Lee, Chun et al. 2005). In one study, treatment of workers exposed to lead with vitamins C and B1 significantly lowered their blood lead levels (Tandon, Chatterjee et al. 2001).

Animal studies suggest that iron supplementation partially reduces the impaired fetal growth caused by cadmium (Cogswell, Weisberg et al. 2003). The essential elements copper, selenium and zinc may also have a significant impact on placental cadmium transport (Zhang, Zhao et al. 2004). In experimental animals, cadmium caused an increase in reactive oxygen species but supplementation with vitamins C and E reduced oxidative stress in the exposed rats (Sen Gupta, Sen Gupta et al. 2004) and rabbits (Beytut, Yuce et al. 2003). Cadmium exposure in rats also appears to inhibit zinc and iron transport from the placenta to the fetus, as well as copper, calcium, sodium and potassium uptake and transportation across the placenta, possibly affecting fetal growth and metabolism (Kuriwaki, Nishijo et al. 2005). Selenium may also play an active role in maternal defence systems against the toxicity of metals and constituents of cigarette smoke (Kantola, Purkunen et al. 2004).

The evidence presented is highly suggestive but not conclusive that low level exposures to heavy metals can affect maternal and fetal health and provides a rationale to test nutritional strategies for mitigating these effects.

6. *Genetic Susceptibility*

a) Metal Toxicity

There is considerable variability in the general population with respect to susceptibility to heavy metal exposure and individual responses may be modulated by genes (Suk and Collman 1998). Genetic differences in the ability of individuals to absorb, distribute, metabolize and excrete toxicants, such as lead, are recognized as potentially important contributors to the large between-individual variation in toxicity. Genetic influences have reportedly accounted for 65% of the variability in blood cadmium and 58% for lead levels in non-smoking women (Bjorkman, Vahter et al. 2000). Recent studies have demonstrated associations with three polymorphic genes that may affect absorption and distribution of lead: aminolevulinic acid dehydratase (ALA-D) coding gene, vitamin D receptor (VDR) gene, and

hemochromatosis (HFE) gene coding for HFE protein (Onalaja and Claudio 2000). A study of genetic effects on blood lead levels in adult twins clearly demonstrated the significant heritability of blood lead concentrations, with an estimated heritability at just over 40% (Whitfield et al., online 2007).

Heavy metals such as mercury and cadmium can induce and bind to metallothionein (MT), a small sulfydryl-rich protein (Kobayashi, Miyagawa et al. 2005). Placentas of smokers have been shown to have higher MT and cadmium levels and been associated with lower birth weights (Ronco, Arguello et al. 2005). While MT over-expression protects against cadmium toxicity, it seems to have no effect on mercury toxicity (Beattie, Owen et al. 2005). Delta-amino-levulinic acid dehydratase (ALAD) is the principal enzyme involved in lead kinetics and carriers with the ALAD-2 allele (Smith, Wang et al. 1995; Perez-Bravo, Ruz et al. 2004; Louis, Applegate et al. 2005) or ALAD 1-1 genotype (Kim, Lee et al. 2004) may be more susceptible to lead toxicity. ALAD enzyme activity is inhibited in maternal blood compared to cord blood (Campagna, Huel et al. 1999), suggesting a potential role for the ALAD gene in the maternal-fetal transfer of lead (Ettinger, et al. 2006 Unpublished data). Other genotypes associated with heavy metal absorption and/or toxicity include hemochromatosis (HFE) (Wright, Silverman et al. 2004), vitamin D receptor (Haynes, Kalkwarf et al. 2003; Chuang, Yu et al. 2004), and apolipoprotein-E (apo-E) (Godfrey, Wojcik et al. 2003). Only a few of the previous studies have evaluated these genotypes in pregnant women or developing infants.

SNPs

- aminolevulinic acid dehydratase (ALAD) 1
- hemochromatosis HFE (C282Y, HFE3', HFE5') 3
- Vitamin D receptor (VDR [fokI, bsmI, apaI, taqI]) 4
- Metallothionein IIA 1
- apolipoprotein E (rs440446, rs405509, rs429358, rs7412) 4

b) Oxidative Stress

Glutathione S-transferases (GSTs) are a family of enzymes important in the biotransformation and detoxification of xenobiotics (Hayes et al., 2005). GST enzymes detoxify reactive oxygen species (ROS) and genetic polymorphisms alter this detoxification pathway resulting in 'oxidative stress' to the cells. The toxic effects of metals are thought to be caused, at least partially, by oxidative stress mechanisms (Ercal et al., 2001;Valko et al., 2005) and early developmental exposure to metals have been shown to suppress xenobiotic-metabolizing enzyme activities (Pillai et al., 2009). Genetic polymorphisms of human glutathione S-transferases (hGSTs), therefore, have important implications for the metabolism and excretion of toxic chemicals, including metals, and may be important in the inter-individual variability in susceptibility to the toxic effects of such exposures. Oxidative stress has been strongly linked with the occurrence of preeclampsia (Gupta et al., 2005) and polymorphisms in maternal biotransformation enzymes have been investigated with respect to risk of preeclampsia (Zusterzeel et al., 2007).

SNPs

- Glutathione-S-transferases (GSTP1 114, GSTP1 105, GSTT1 224, GSTT1 5'UTR, GSTM1 173, GST01 140, GST01 208) 7
- □ Glutathione peroxidase (GPX1 198) 1

c) Pregnancy Induced Hypertension & Preeclampsia

(1) Endothelins

Endothelins are potent vasoactive peptides. Endothelin levels are elevated in a number of cardiovascular diseases, and the endothelin system may play an important role in preeclampsia. There is a body of literature showing an association between endothelin gene variants and blood pressure and blood pressure reactivity (Banno et al., 2007;Barden et al., 2001;Moore et al., 2004;Rahman et al., 2008). Furthermore, polymorphisms in other genes have been shown to impact circulating endothelin levels. For example, carriers of the chromogranin promoter minor alleles -988G, -462A, and -89A had significantly higher mean plasma ET-1 (Lillie et al., 2007). At the same time, it has been shown that contaminants can alter endothelin levels. Circulating levels of endothelin-1, endothelin-2, and endothelin-3 are increased after exposure to air pollution (Bouthillier et al., 1998;Calderon-Garciduenas et al., 2007;Elder et al., 2004;Thomson et al., 2005;Vincent et al., 2001) indicating that environmental contaminants can alter endothelin homeostasis. These studies indicate that assessment of polymorphisms in endothelin family genes may be critical to interpreting variations in endothelin levels and their physiological consequences in response to contaminant exposure.

SNPs

- Endothelin-1 (K198N) 1
- Endothelin-2 (A985G) 1
- Chromogranin A (T988G, G462A, C89A) 3

(2) MTHFR (Methylenetetrahydrofolate reductase)

Reduction in MTHFR enzymatic activity would result in elevated levels of plasma homocysteine, a risk factor for venous thrombosis. In preeclampsia, reduced placental perfusion and endothelial cell dysfunction are noted. Increased plasma homocysteine may play a role in the etiology of preeclampsia. Recently, Braekke et al. (2007) found that median concentrations of plasma homocysteine, cysteine, choline and betaine were elevated, both in maternal and fetal compartments in preeclampsia pregnancies, compared with normal pregnancies.

However, the association between the polymorphism of the MTHFR gene and the prevalence of preeclampsia is still controversial. One explanation could be the variation of the definition for preeclampsia. Conversely, ethnic differences have been proposed as factors in genetic susceptibility (Cummings and Kavlock, 2004).

Currently, a total of 41 rare but deleterious mutations in *MTHFR*, as well as about 60 polymorphisms have been reported (Leclerc and Rozen, 2007). Two *MTHFR* variants, however, the C677T and the A1298C mutations, are common in many populations. The C677T allele is a single base pair mutation in which a cytosine is converted to a thymine at basepair 677, resulting in an amino acid substitution (alanine to valine) in the enzyme. In the A1298C allele, a point mutation in exon 7 results in the coding of a glutamate instead of an alanine residue. This allele has also been termed C1289A mutation by other authors.

Homozygotie 677TT frequency is about 12% in Canada and 14% in Quebec (Leclerc and Rozen, 2007). To date, data on the prevalence of the A1298C allele in the population is limited to relatively

small groups of controls from case-control studies. The frequency of A1298C homozygotes among controls was approximately 9% in two studies, one from Canada (Weisberg et al., 1998) and one from the Netherlands (van der Put et al., 1998).

Several authors indicate an association between homozygotie for 677T allele and the incidence of preeclampsia. The functional repercussions of the other polymorphisms were little characterized or remain unknown.

SNPs

- Methylenetetrahydrofolate reductase - MTHFR (677CT, 1298AC, Gln594Arg) 3
- Methyltransferase reductase - MTRR (66AG) 1
- Methltransferase - MTR (2756AG) 1
- Transcobalamin - TCN2 (776CG) 1

d) Pre-term Birth

Preterm birth is a complex trait and possesses the following features: non-mendelian transmission, the involvement of multiple genes, and gene-gene and gene-environment interactions. Research on the genetics of preterm birth thus faces significant challenges. Among the potential candidate genes affecting preterm birth through the endocrine pathway are the Estrogen receptor α, the Estrogen receptor β, the Progesterone receptor, and the CYP19 gene. The CPY19 codes for the enzyme aromatase that is responsible for the conversion of androstenedione to estradiol in the placenta. CYP19 may also be important in the metabolism of endocrine disrupting chemicals (Hakkola et al., 1996;Pasanen, 1999). It has been reported that Mono-(2-ethylhexyl) phthalate (MEHP) dose-dependently suppressed aromatase activity and its transcription level (Noda et al., 2007). Single nucleotide polymorphisms (SNPs) in the human estrogen metabolism and estrogen pathway have been associated with adverse pregnancy outcomes such as miscarriage (Cupisti et al., 2009). Associations between SNPs in the progesterone receptor and preterm birth have also been reported (Diaz-Cueto et al., 2008;Guoyang et al., 2008). It is plausible that endocrine disrupting chemicals interact with these genes as a mechanistic pathway for preterm birth. Moreover, this analysis could help to understand variations in sensitivity to endocrine disruptors among pregnant women.

SNPs:

- Cytochrome P450 (UTR CT, UTR AC) 2
- Progesterone receptor (770[C>T] or H770H, 660[G>T] or V660L) 2
- Estrogen receptor α (intronic nucleotide exchange CT) 1

7. Prenatal Exposure to Other Priority Environmental Contaminants and Tobacco Smoke

In Canada, there is no national biomonitoring program in place to measure exposure of the general population or pregnant women in particular to heavy metals or other environmental contaminants. While it is expected that Health Canada's partnership with Statistics Canada's Canadian Health Measures Survey (CHMS) (2007 - 2009) will provide much needed national data on exposure of the Canadian population to several priority environmental contaminants, it will not collect data for two of the most susceptible and vulnerable populations - the pregnant woman and her fetus. Based on both internal and external consultations, Health Canada identified a list of priority environmental chemicals for biomonitoring. A sub-set of potentially endocrine-modulating chemicals will be measured in both MIREC and the CHMS by the same analytical laboratory (brominated flame retardants (PBDEs), persistent organochlorine pesticides, PCBs, plasticizers (phthalates and bisphenol A), smoking by-products (cotinine), organophosphate pesticides and surfactants (perfluorinated compounds)).

The persistent and bioaccumulative environmental chemicals have been detected in maternal blood and urine, cord blood and placental tissues, even in areas remote from local point sources of emission (Adibi, Perera et al. 2003; Guvenius, Aronsson et al. 2003; Mazdai, Dodder et al. 2003). Recently, concern about the health effects of exposure to brominated flame retardants (PBDEs) and plasticizers (phthalates and bisphenol A) has been raised based on research showing trends of increasing body burdens in selected populations, particularly women of reproductive age, infants and children. Human and toxicological evidence is emerging about potential health effects of these chemicals, especially when exposure occurs during pregnancy. Of particular concern are endocrine-modulating effects such as associations between maternal phthalate exposure and effects in genital development in male infants (Swan, Main et al. 2005), prenatal exposure to bisphenol A and altered brain sexual differentiation in mice (Rubin, Lenkowski et al. 2006) and thyroid function in rats (Kobayashi, Miyagawa et al. 2005), and effects on male fertility and neurobehavior in rat offspring exposed to PBDEs (Kuriyama, Talsness et al. 2005). More recently, perfluorinated compounds, an emerging class of persistent organohalogen chemicals, have been detected in human sera and liver of non-occupationally exposed adults (Hansen, Clemen et al. 2001; Olsen, Hansen et al. 2003) and in tissues of children (Olsen, Burris et al. 2002). In rat and mouse models, exposure to high levels of one of the most widespread perfluorinated compounds (PFCs), perfluorooctane sulfonate (PFOS), has been reported to impart toxicity during pregnancy. Maternal effects included reduction in body weight gain associated with a reduction in water and food intake, and fetal effects included reduction in fetal weight, bone dysfunction, edema and cleft palate (Lau, Butenhoff et al. 2004). Maternal exposure to low PFOS levels also resulted in reduced maternal body weight at termination and reduced weight gain. This was particularly evident when PFOS exposure was accompanied by physical stress (Fuentes, Colomina et al. 2006). I*n utero* exposure to perfluorinated chemicals have also caused post-natal rat pup mortality (Luebker, Case et al. 2005). The estimated half-lives of PFCs in adult workers ranges from 4 to 9 years (Olsen et al., online 2007).

Prenatal exposure to organophophate pesticides has recently been associated with impaired fetal growth (Whyatt et al., 2004; Perera et al., 2003), abnormal neonatal behaviour and primitive reflexes (Engel et al., 2007) and pervasive developmental problems at 24 months of age (Eskenazi et al., 2007). The only recent biomonitoring study of exposure to organophosphate pesticides in Canada was conducted in Quebec children and reported higher levels than those observed in the US studies (Valcke et al., 2006).

Smoking during pregnancy has long been associated with low birth weight, fetal and infant mortality, congenital malformations, infertility and long-term effects on the child (U.S.-Department-of-Health-and-Human-Services 1989). The U.S. Surgeon General concluded that children of parents who smoke have a greater risk of suffering respiratory diseases and reduced lung function. Studies have shown that exposure to environmental tobacco smoke (ETS) during pregnancy can result in an increased risk of low birth weight (1.5 - 4 times more likely) (Fortier, Marcoux et al. 1994; Misra and Nguyen 1999) and an increased risk of central nervous system (CNS) tumours among the children of non-smoking mothers exposed regularly to ETS in pregnancy (increased risk of around 80%) (Filippini, Farinotti et al. 2000). Although smoking has been consistently associated with poor fetal growth or low birth weight, ironically, smoking women seems to have a lower risk of preeclampsia (which is a common condition associated with poor fetal growth) - the complex effects and pathways of tobacco by-products on pregnancy outcomes remain to be fully understood.

In Canada, in 2003, 25% of Canadian women aged 20- 44 reported being pregnant in the last five years. Of these, 12% smoked regularly during their most recent pregnancy. This is down from 19% as

reported in the 1995 Survey on Smoking in Canada. As well, in 2003, 12% said that their spouse smoked regularly at home during their most recent pregnancy (Health-Canada 2003). There are indications that some women continue to smoke in order to deliver a lower birth weight infant to make birthing easier. Other reports indicate that women who quit smoking during pregnancy resume smoking after delivery. In addition to data gaps with respect to smoking behaviour and exposure to tobacco smoke by expectant mothers, there is very little evidence regarding pre-, during and post-pregnancy smoking behaviour of individual smokers as well as transmission of the harmful ingredients in tobacco smoke to infants.

There are several key indicators of exposure to tobacco products, including nicotine, and its metabolite, cotinine. Tobacco is a major source of heavy metals (lead, cadmium and mercury) for smokers (U.S.-Department-of-Health-and-Human-Services 1989; Rickert and Kaiserman 1994). According to a Canadian Environmental Act Assessment on cadmium, for smokers, smoking is the second major source of cadmium after all foods and is equal to about one-quarter of food ingestion for adults and slightly greater than one-fifth for adolescents (CEPA-(Canadian-Environmental-Protection-Act) 1994).

Given that tobacco smoke is a major source of many environmental contaminants, the measurement of levels of environmental contaminants in blood and urine (i.e. biomonitoring) and apportionment of these contaminants will be an important tool to assess human exposures and health risk. Analysis of these metals, in conjunction with self-reported behaviour, knowledge of smokers' brands and knowledge of the emissions of heavy metals from these brands, could provide source apportionment and a basis of comparison of body burdens between smokers, non-smokers and passive smokers, and disentangle the proportion of exposures from tobacco smoking versus other environmental exposures. Such information is valuable but lacking.

8. Vitamin D Assessment

Vitamin D is a hormone synthesized endogenously as a result of skin exposure to ultraviolet beta (UVB) radiation and is obtained as a nutrient through foods/supplements. Endogenous synthesis accounts for the majority of vitamin D in adults. Above the 42nd parallel N, synthesis is limited to late spring through mid-autumn, because of the low intensity of UVB radiation, during the rest of the year.. The majority of Canadians reside north of the 42nd parallel, placing them at risk for hypovitaminosis D year round. Use of sunscreen and environmental factors, such as clothing and pollution, reduce exposure to UVB sunlight in summer. This means that endogenous synthesis in pregnant women and subsequent maternal-fetal transfer is likely to be compromised. Fortified foods such as milk, margarine and vitamin supplements remain the major source of vitamin D and natural foods contain only very small amounts and significant sources for vitamin D are confined to a very limited number of animal products such as fish oils, fatty fish, eggs, and liver

Given the fact that Canadian women do not consume enough milk for reasons which may include financial barriers, food allergies or cultural considerations, consuming on average 1.6 servings per day, it is not surprising that over 1/3rd of infants are born already deficient in vitamin D. In Manitoba, Dr. Weiler has documented that 36% of infants from white or non-white parents are deficient in vitamin D at birth, defined as a serum 25(OH)D below 27.5 nmol/L, with 46% of their mothers themselves having values < 37.5 nmol/L. Additional data confirming low vitamin D status in Canadian women have demonstrated that 34 % of women in Calgary have low vitamin D status in at least one season, and that in Toronto, 14.8 % of white women and 25.6 % of non-white, non-black women are deficient. These high rates of deficiency occurred despite daily consumption of 200-400 IU of vitamin D. Therefore, it can be expected that many Canadian newborn infants will have low vitamin D status as a result. These

studies suggest that vitamin D deficiency is not uncommon, particularly in non-white women and their infants. Likewise season of birth and ethnicity are important factors to consider in defining how much vitamin D is required to achieve and maintain target values for 25(OH)D in infants.

The effects of higher maternal-fetal transfer of vitamin D are sustained long into childhood. Maternal vitamin D status in pregnancy is positively correlated with bone mass in the children measured as late as 9 years of age. Vitamin D early in life is also linked to brain growth in animals and schizophrenia. In adults, vitamin D is under study as a therapy for multiple sclerosis and vitamin D deficiency also considered as a possible cause of certain cancers.

A large scale study to determine the prevalence of vitamin D deficiency in pregnancy women and newborn infants has never been conducted in Canada. Given the current concerns about vitamin D status of Canadians, this study will be very significant and important to public policy and research agendas in achieving and maintaining health.

> Vitamin D status is currently judged by serum 25(OH)D and the parathyroid hormone (PTH)-25(OH)D dynamic. The ultimate effect of vitamin D on human health is a healthy skeleton, which is characterized in infancy by normal linear growth and bone mineral accretion and absence of bone related disease, such as rickets. The importance of these indicators is illustrated in epidemiological research, where linear growth in infancy is positively related to peak bone mass as an adult. Moreover, both vitamin D status and intake in infancy are related to bone mass in children. Conversely, vitamin D deficiency causes rickets in infants and children. Both congenital rickets and rickets in infancy continue in Canada as reported by the Canadian Pediatric Surveillance System.

Both Health Canada and Institute of Medicine acknowledge that there is considerable uncertainty regarding what defines optimal vitamin D intake in pregnancy and infancy based on functional outcomes. The uncertainty stems from lack of studies that include multiple dosages of vitamin D and also lack of comprehensive assessment, including assessment of dietary intakes of vitamin D and vitamin D status as judged by 25(OH)D-PTH dynamic data.

In order to better understand how to optimize vitamin D status in pregnant women and infants, biomarkers of bone formation and resorption should be included to more comprehensively assess bone metabolism in association with 25(OH)D and PTH. Suggested biomarkers include plasma osteocalcin and urinary N-telopeptide. Osteocalcin increases with growth and bone mineralization in the first year of life, as does alkaline phosphatase. Osteocalcin is the superior marker, since it also reflects seasonal changes in 25(OH)D and PTH in infancy. For bone resorption, N-telopeptide is a practical marker, as it can be measured in plasma or urine and is also a specific index for bone resorption. In pregnant women these biomarkers will reflect changes in bone metabolism with vitamin D and PTH as well as reflect adaptation to pregnancy state to enable maternal-fetal transfer of mineral.

OBJECTIVES:

1) to determine vitamin D status of pregnant mothers and that of their newborn infants;
2) establish the proportion of pregnant mothers with vitamin D deficiency in early and late pregnancy and to establish the proportion of newborn infants with vitamin D deficiency;
3) determine 25(OH)D-PTH dynamics in early and late pregnancy and in cord blood in a sample of pregnant women spanning Canada coast to coast;

4) to establish if biomarkers of bone formation and resorption improve interpretation of vitamin D status in pregnant women and newborn infants; and
5) determine seasonal variations in vitamin D status in pregnancy women and newborn infants; and
6) estimate exposure to sunshine as a covariate in assessment of vitamin D status in pregnant women.

9. Lactational Exposure: Heavy metals and Other Priority Environmental Chemicals

No national biomonitoring studies which include both pre- and post-natal exposure measurements on the same individuals have ever been conducted. Human milk is an integral part of the food-chain for a vulnerable population and thus can be used to obtain information related to infant exposure (Needham and Wang 2002). Infant toxicokinetics has also been identified as a gap in our understanding of the fate of environmental chemicals (LaKind, Brent et al. 2005). While human milk biomonitoring does not provide data on absorption, distribution, metabolism and elimination (essential elements in toxicokinetics) by the infant's body, the information generated from biomonitoring can assist in the process insofar as providing the range of external doses of infants in the general population (LaKind, Berlin et al. 2001; LaKind, Brent et al. 2005).

Since 1967, Health Canada has conducted five human milk surveys (in 1967, 1975, 1981, 1986, and 1992) to determine the concentrations of persistent chlorinated pesticides and industrial organic contaminants, now commonly referred to as persistent organic pollutants (POPs). Only one survey, that of 1981, included human milk analyses of lead and cadmium.

Time-trend analysis of the previous national surveys showed that DDTs, the most predominant group of contaminants in human milk displayed the most consistent decline since 1967. Total PCBs and hexachlorocyclohexane peaked in 1982 and then abruptly decreased to pre-1975 levels (Mes 1994; Newsome, Davies et al. 1995; Craan and Haines 1998). Dioxins and furans were incorporated in the analysis as of 1981. Between 1981 and 1986, the levels dropped by nearly half and remained constant through 1992 (Ryan, Lizotte et al. 1993). Retrospective analysis of PBDEs in stored human milk showed a doubling in concentration over a 10 year period, from 1992 to 2001 (Ryan and Patry 2001). Table 1 provides the trend for various POPs.

Table 1. Trends of average POPs residues, ng/g whole milk in Canadian human milk. Dioxin/furan WHO Toxic Equivalents (TEQ) are expressed in ng/kg.

Compound	Year of sample collection					
	1967	1970	1975	1981	1986	1992
PCBs		6	12	26	6	7
HCB			2	2	1	<1
β-HCH			2	8	1	0.71
γ-HCH	3	2		trace	<1	<1
Heptachlor epoxide	3	4	1	trace	<1	<1
Oxychlordane			1	1	1	<1
Trans-nonachlor			1	1	1	1
Dieldrin	5	5	2	1	1	<1
DDE		57	35	34	10	7
Dioxin/Furan TEQ				0.89	0.56	0.52
Fat content (%)	2.66 (0.4-7.4)	NA	2.20 (0.2-7.3)	3.82 (0.5-9.1)	3.58 (0.1-19.4)	3.32 (0.1- 13.5)

Associations between levels of the residues and various independent factors were established as a result of these surveys. First, concentrations of POPs were found to be consistently higher in milk of primiparous women as compared to multiparous women (Newsome, Davies et al. 1995; Craan and Haines 1998). This indicated that first-borns of lactating mothers may be exposed to a greater extent than any subsequent child born of the same woman, provided there is little variation in daily milk intake and length of lactation (Mes, Davies et al. 1993; Newsome, Davies et al. 1995). Maternal age was also postulated to positively correlate with maternal body burden. Results from the Canadian human milk surveys did not show a clear and direct increase of maternal body burden with increasing age. Rather, the relation of maternal age and residue levels was shown to be modified by parity. When the effect of parity was removed by considering only primiparous women, the median residue level did increase numerically but not statistically between age groups. When the data were adjusted for fat content, DDE, trans-nonachlor and total PCBs did increase statistically with age (Newsome, Davies et al. 1995). This pattern is similar to other published reports on the effect of parity and age on residue concentrations (Jensen and Slorach 1990).

Analysis of heavy metals from the 1981 survey showed that the levels of lead in the milk were influenced by the age of the house the participant lived in, and the long-term (greater than 5 years) exposure of the mother to heavy traffic. As for cadmium, the analysis confirmed that smoking was the chief source of exposure and smoking was statistically correlated with levels of cadmium in breast milk (Dabeka, Karpinski et al. 1986).

Despite the detection of the POPs and heavy metals in mother's milk, Health Canada has consistently concluded that the nutritional, psychological and immunological benefits provided from human milk

to the child outweigh the health risks from exposure to these contaminants (Mes 1994; Newsome, Davies et al. 1995; Craan and Haines 1998). This is supported by prospective studies that were conducted on non-representative cohorts in the Netherlands and in the USA. Their results showed that the associations between prenatal exposure to PCBs and poorer cognitive performance in childhood were generally stronger and statistically significant only among non-breast-fed children (Patandin, Lanting et al. 1999; Jacobson and Jacobson 2002). While it remains uncertain whether the benefits were derived from optimal nutrient intake, optimal parental stimulation or both, breast feeding was concluded to outweigh the alternative and potentially provided protection against contaminant exposure that might have occurred *in utero*.

10. Lactational Exposure: Nutrients and Immunoprotective constituents

Human milk contains many nutritional substances deemed critical for optimum infant development (LaKind, Amina Wilkins et al. 2004; LaKind, Brent et al. 2005). Changes in milk composition over the course of lactation, from colostrum to mature milk, ensure optimum nutrition to the growing child. Infant formula has been modified over time to more closely resemble human milk, but still does not contain many of the substances important for optimal infant health and development (LaKind, Brent et al. 2005). Using the example of fatty acids, vitamin D and immunoprotective constituents, the following three paragraphs will serve to illustrate the importance of national-level human milk biomonitoring for beneficial components in conjunction with environmental contaminants.

The composition of fatty acids consumed by infants through breast milk is of concern because of the important roles of long chain polyunsaturated fatty acids for optimum infant development and because of the transfer of potentially detrimental *trans* fatty acids (Innis and King 1999). The majority of the *trans* fats in our diet are industrially produced and are typically found in foods made with partially hydrogenated oil, primarily baked and fried foods (Trans-Fat-Task-Force 2006). *Trans* fatty acids have been linked with cardiovascular disease (Mozaffarian, Katan et al. 2006) and are reported to adversely affect the metabolism of beneficial long chain polyunsaturated fatty acids, particularly in infants (Innis and King 1999). Earlier studies have already detected *trans* fatty acids in Canadian human milk and indicated that Canadian milk has higher levels than do European human milk samples at the time of the survey (Chen, Pelletier et al. 1995; Innis and King 1999). Further, levels of fatty acids in human milk are reported to be influenced by maternal diet and are positively correlated with plasma levels of breastfed infants (Innis and King 1999). Recently, recommendations were proposed by the Trans Fat Task Force (Trans-Fat-Task-Force 2006) to significantly reduce levels of *trans* fats in processed foods sold in Canada. National-level biomonitoring of human milk for a complete fatty acids profile (including *trans* fats) would allow time trend analyses and could serve as baseline data for future evaluation of the effectiveness of the recommendations put forth by the Trans Fat Task Force.

Vitamin D, a lipid soluble vitamin, performs an important function in bone metabolism in that it influences active calcium absorption at the intestines. The relative amount of active vitamin D will depend on the extent of the individual's dietary intake and extent of sun exposure. Because dietary sources of vitamin D are limited to fatty fish and in some meats (Molgaard and Michaelsen 2003), human milk typically contains small amounts of vitamin D and children who are breastfed and do not receive supplementation or sun exposure are at high risk of developing vitamin D deficiency (Dawodu, Agarwal et al. 2003). The health implications of vitamin D deficiency extend beyond rickets, with evidence for vitamin D insufficiency (lesser degree of vitamin D deficiency) playing a role in pediatric and/or adult osteoporosis and osteomalacia, insulin-dependent diabetes, multiple sclerosis, cardiovascular diseases and certain cancers (Holick 2005). It has been suggested from epidemiological data and very limited numbers of small clinical studies carried out to date, that

vitamin D status in children could play a role in the imprinting of future health/disease outcomes such as Type 1 diabetes and asthma (Hypponen, Laara et al. 2001; Harris 2005). In Canada, parents are recommended to provide supplementation to their breastfed infants (Health-Canada 2004). In this context, national-level vitamin D data in human milk will serve to document the current range of levels and to examine the association between levels in human milk and key factors such as sun exposure, supplementation and diet.

Human milk also provides a number of bioactive factors that are vital for protecting newborns in the early postnatal period when the mucosal and systemic immune systems are still developing (Kelleher and Lonnerdal 2001). These endogenous constituents include immunomodulatory, anti-microbial and anti-inflammatory agents that are protective for both the newborn and for the mammary gland itself. A recent study indicated that maternal smoking was associated with altered proinflammatory cytokine IL-1alpha levels in human colostrum milk (Zanardo, Nicolussi et al. 2005). In addition, maternal stress and fatigue were shown to correlate with altered secretory IgA and prolactin levels in human milk (Groer, Davis et al. 2005). These studies highlight the putative link between maternal health, environmental chemical exposure and levels of endogenous milk immunomodulatory constituents.

Given that human milk is the predominant and often the sole-source of food for infants, reference standards for definition of infant nutritional needs are based on levels in human milk. The national scope of the study provides superior "baseline" data to existing published data which are limited in numbers of subjects and/or geographic range. It will also provide the unique opportunity to investigate the influence of various factors such as maternal diet, maternal age, parity, socioeconomic status and other relevant factors on levels of nutrients and immunoprotective constituents.

Further, national-level human milk monitoring that includes concurrent measurements of benefits and risks have yet to be conducted. The results will provide a foundation to study associations between human milk contaminant levels and levels of nutrients and immunomodulatory constituents which may influence maternal and neonatal status and health outcomes. For example, recent but controversial data suggest that perchlorate (a chemical used mostly in rocket fuels) in human milk is negatively associated with levels of iodide in human milk (Kirk, Martinelango et al. 2005). Further, bone resorption, which is suggested to occur during pregnancy, might continue to occur but to a lesser degree during lactation, resulting in continued lead exposure to the breastfed infant (Ettinger, Tellez-Rojo et al. 2004). This was described through an observation of a positive correlation between lead and calcium in whole human milk (Anastacio Ada, da Silveira et al. 2004).

Overall, the results of a study that includes contaminants, nutrients, and immune constituents are expected to strengthen health benefits:risk assessments in infants and to support measures to reduce release of contaminants into the environment, and limit exposure of the general population (Pronczuk, Akre et al. 2002; LaKind, Brent et al. 2005). Furthermore, the Stockholm Convention on POPs whose primary objective is to reduce the amount of 12 priority POPs in the environment and in people, was ratified in 2004 (UNEP-(United-Nations-Environment-Programme) 2004). One of the recommendations of this Convention is to objectively assess the levels of POPs in people so that each country can better identify and prioritize POPs for remedial action. This national-level prospective biomonitoring initiative that includes prenatal and lactational exposure to POPs on the same individuals contributes to our commitment to this landmark treaty.

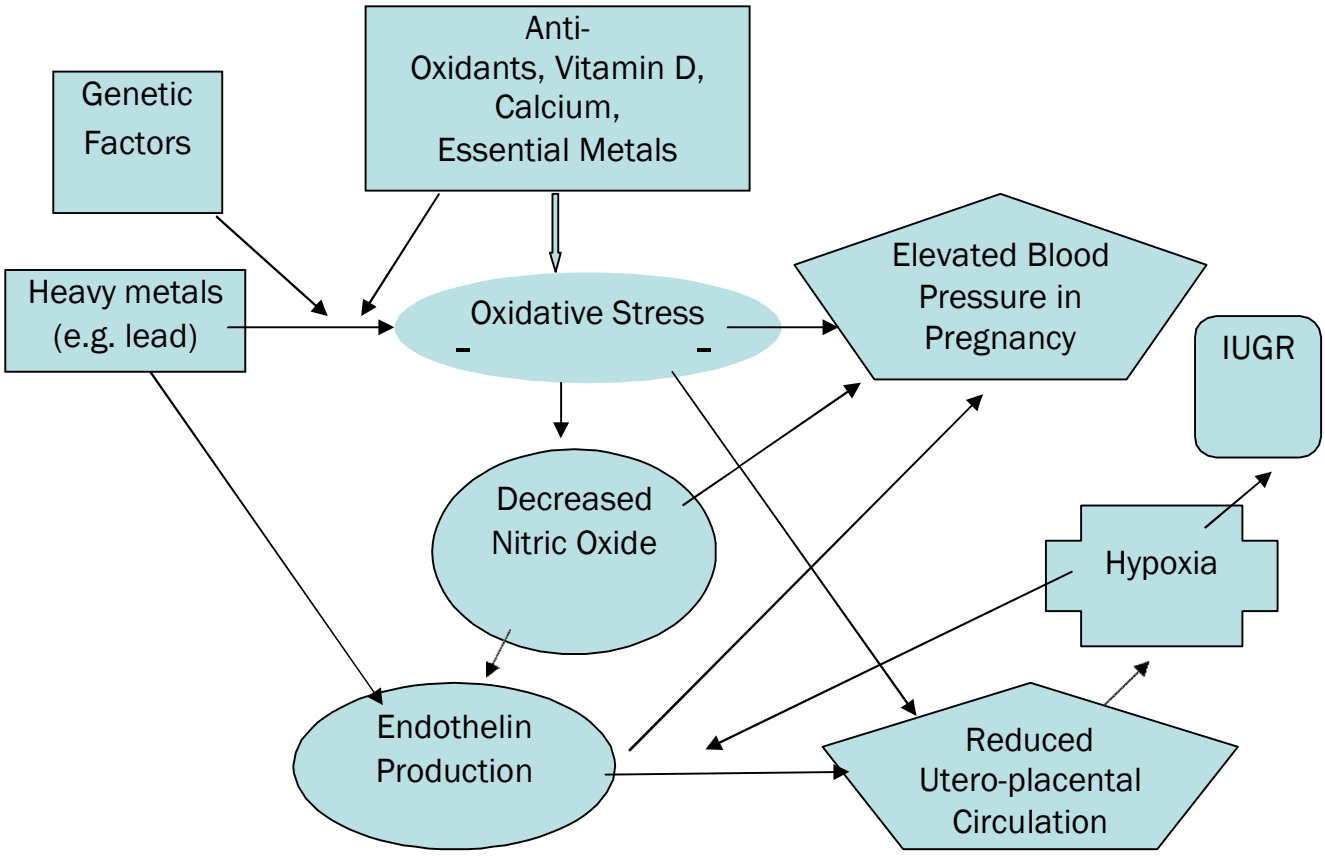
II - Proposal

We propose to establish a new national-level pregnancy cohort with a focus on collecting national level data on exposure to priority environmental chemicals, using both questionnaire-based and biological marker-based approaches. This pregnancy cohort will be created to collect maternal blood and urine samples and questionnaire-based information during scheduled clinical visits, as well as meconium and umbilical cord blood samples at delivery and breast milk and maternal hair post-natally. These specimens will be analysed to estimate maternal and infant body burdens of various environmental chemicals of concern (nicotine, cotinine, heavy metals, POPs, brominated flame retardants, surfactants, some pesticides and plasticizers) as well as nutritional and immunoprotective constituents in human milk. Risks of adverse pregnancy outcomes will be assessed for exposure to heavy metals. The association between human milk contaminant levels and levels of milk immunoprotective constituents and nutrients will be examined and a comprehensive benefits:risk assessment will be undertaken. Appendix 1 presents the 5-year budget based on committed funds.

A - Specific Aims and Hypotheses

The proposed model for the interactions between heavy metals, nutritional status and genetic susceptibility on pregnancy outcome is shown in Figure 1.

Figure 1: Proposed Model of Role of Heavy Metals, Nutrition and Oxidative Stress in Pregnancy Outcomes



In this cohort study, we will test the following hypotheses:

Primary Hypothesis:

- ☐ Elevated maternal body burdens of heavy metals (i.e., lead, cadmium, manganese, mercury, arsenic) are associated with increased maternal blood pressure and altered fetal growth.

Secondary Hypotheses

- ☐ Higher calcium intake reduces maternal bone resorption and therefore the mobilization of lead and other heavy metals from maternal bone and the resultant association between body burdens and blood pressure or fetal growth retardation.
- ☐ Heavy metal exposure causes oxidative stress and increases the production of endothelins leading to reduced utero-placental circulation resulting in fetal growth retardation.
- ☐ Carriers of specific genes are at increased risk of heavy metal toxicity.
- ☐ Higher environmental contaminants load in human milk adversely affect immunoprotective constituents and nutrients in human milk.

Primary Objectives

- ☐ To determine whether contemporary non-occupational-level heavy metal exposure as measured by maternal and fetal body burdens is related to elevated maternal blood pressure, hypertension and fetal growth retardation.
- ☐ To obtain national-level data on maternal and neonatal exposure to priority environmental contaminants.
- ☐ To obtain Canadian data on smoking behaviour and exposure to tobacco smoke (active and passive) in pregnancy.
- ☐ To obtain contemporary levels of priority environmental chemicals (selected POPs, heavy metals, mycotoxins, perchlorate), food packaging and food processing related chemicals (bisphenol A, phthalates, hetereocyclic aromatic amines), selected nutrients (priority vitamins, complete fatty acids profile (including trans and omega-3 fatty acids), priority and other relevant minerals) and relevant immunoprotective endpoints and antioxidative markers in mature human milk
- ☐ To obtain contemporary levels of maternal hair-mercury.
- ☐ To characterize dietary exposure of breastfed infants ages 3-8 weeks.
- ☐ To allow for time-trend analyses for those analytes which were included in previous human milk surveys.

Secondary (Exploratory) Objectives

- ☐ To measure the distribution of pre- and post-natal body burdens of heavy metals in a population of Canadian women over the course of pregnancy, examine the correlation between maternal and fetal body burdens, and identify factors that affect the concentration of heavy metals in newborn infants (as measured by cord blood and meconium analyses).
- ☐ To investigate possible avenues for secondary prevention against metal-induced toxicity (e.g., antioxidant vitamins, calcium, selenium).
- ☐ To elucidate the oxidative stress pathways by analysing specific metabolic biomarkers and examining their association with heavy metal concentrations in maternal blood as well as associations with vasoregulatory components such as the plasma endothelins and free nitrite levels.
    - markers of oxidative stress include:
        - plasma isoprostane and tyrosines (p-,m-,o-,Cl-, 3-nitro), uric acid, L-DOPA, 8-hydroxydeoxyguanosine and norepinephrine.
    - vasoregulatory components include:

  - circulating plasma endothelins (big-ET1, big-ET2, big-ET3) and corresponding mature peptides ET1, ET2 and ET3.
  - free nitrite levels in plasma.

☐ To explore candidate genetic polymorphisms that may explain differences in susceptibility to metals toxicity, and certain pregnancy outcomes such as preeclampsia and preterm birth; genes that code for:
  - aminolevulinic acid dehydratase (ALAD)
  - apolipoprotein E family (rs440446, rs405509, rs429358, rs7412)
  - hemochromatosis HFE (C282Y, HFE3', HFE5')
  - Vitamin D receptor (VDR [fokI, bsmI, apaI, taqI)
  - Metallothionein IIA
  - Glutathion-S-tranferases (GSTP1 114, GSTP1 105, GSTT1 224, GSTT1 5'UTR, GSTM1 173, GSTO1 140, GSTO1 208)
  - Glutathione peroxidase (GPX1 198)
  - Methylenetetrahydrofolate reductase MTHFR (677CT, 1298AC, Gln594Arg)
  - Methyltransferase reductase MTRR (66AG)
  - Methyltransferase MTR (2756AG)
  - Transcobalamin TCN2 (776CG)
  - Endothelin-1 (K198N)
  - Endothelin-2 (A985G)
  - Chromogranin A (T988G, G462A, C89A)
  - Cytochrome P450 (UTR CT, UTR AC)
  - Progesterone receptor (770[C>T] or H770H, 660[G>T] or V660L)
  - Estrogen receptor α (intronic nucleotide exchange CT)

☐ To identify environmental and maternal dietary and lifestyle factors which correlate with levels of nutrients, environmental chemicals and immunoprotective constituents in human milk.

☐ To examine the correlations between environmental chemicals and nutrients in human milk.

☐ To examine the correlations between environmental chemicals and immunoprotective constituents in human milk.

☐ To undertake a comprehensive risk:benefits analysis for human milk.

B - Research Design and Methods

11. Study Population

Clinical site investigators in Vancouver, Edmonton, Winnipeg, Sudbury, Toronto, Hamilton, Kingston, Ottawa, Montreal, and Halifax have been invited to recruit women attending their clinics during the first trimester of pregnancy (6 – 13^{+6} weeks). Eligibility criteria include ability to consent and to communicate in English or French, age 18 years or older, planning on delivering at a local hospital, and agreeing to participate in the cord blood collection component of the MIREC study. Women with the following medical history will be excluded from the study:

☐ Women who have known foetal abnormalities (e.g. hydatidiform mole), or known fetal chromosomal or major malformations in the current pregnancy.

☐ Women who have a history of medical complications including:
  - renal disease with altered renal function
  - epilepsy
  - any collagen disease such as lupus erythromatosus and scleroderma
  - active and chronic liver disease (hepatitis)
  - heart disease
  - serious pulmonary disease

- cancer
- haematologic disorder (patient with anaemia or thombophylias will be included)
- threatened spontaneous abortion. Women with previous bleeding in the first trimester, can be included if the site documents a viable fetus at the time of recruitment
- illicit drug use.

12. Overview of Recruitment and Collection

Research nurses from all clinic sites will be trained at a central location in patient screening, recruitment, consenting, specimen and data collection and processing, as well as shipping biospecimens.

A poster and pamphlet have been developed to facilitate recruitment of pregnant women to the study (see Appendices 6 & 7). The pamphlet will contain the contact information for the city in which it is displayed. These recruitment materials will be placed in physician offices and other locations identified by the clinic sites. A communications strategy is also being developed to communicate information about the study to appropriate levels of governments and non-governmental agencies, as well as the public.

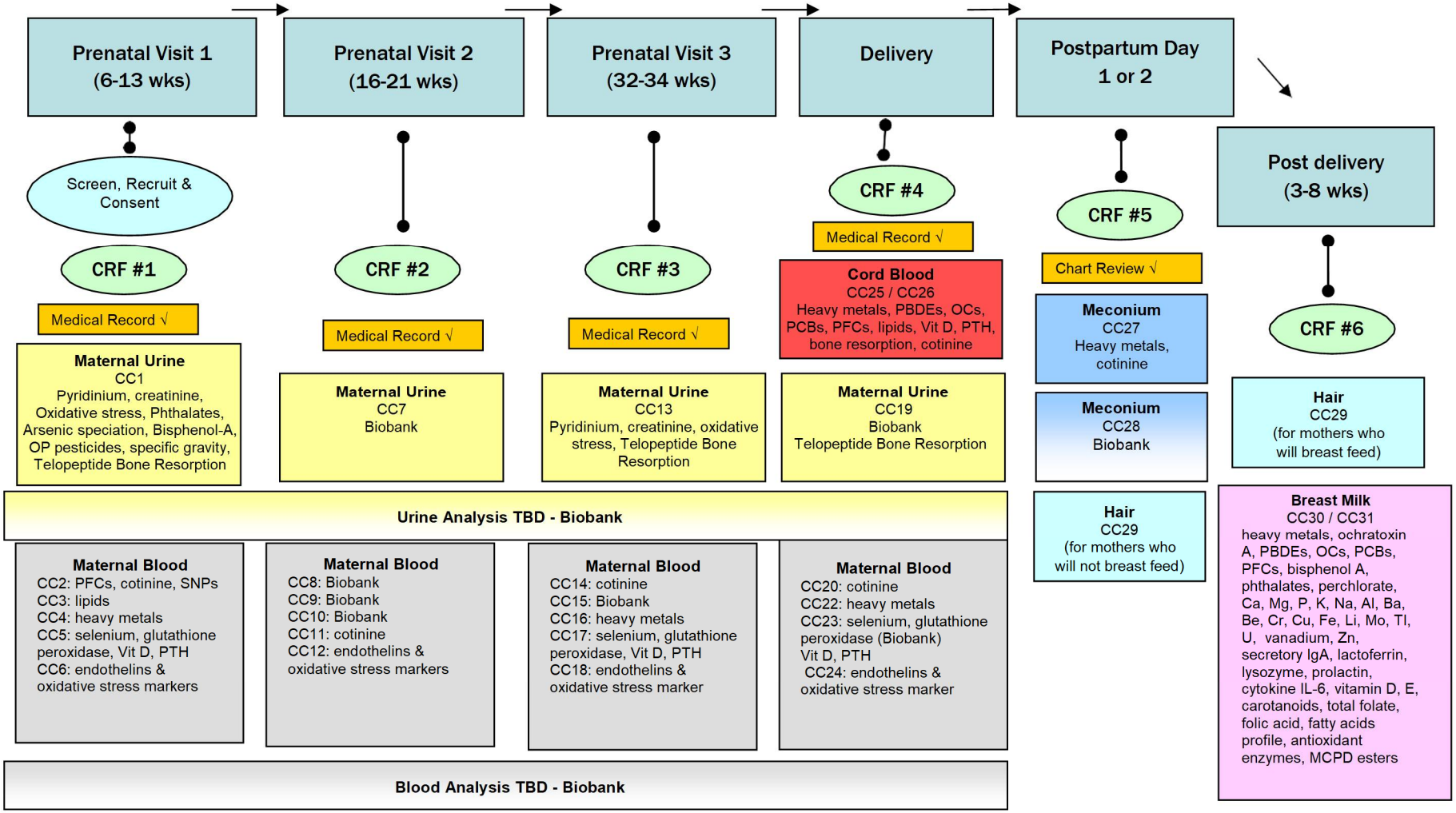
The goal is to recruit women that are representative of the population of pregnant women in their area over a 2 to 3 year recruitment period, with each site to recruit participants until the target of 2,000 is reached by the fall of 2010.

The research nurse will explain the requirements of the current study to potential participants and will administer a screening questionnaire (see Appendix 2). When a patient accepts to participate in the current study and is eligible, written consent (see Appendix 3) must be obtained from her prior to her being entered into the study. A separate consent form for the use of the stored biological specimens collected for future research will also be obtained. Copies of the signed consent forms will be given to the woman for her records.

Maternal blood, umbilical cord blood, urine, infant meconium, human milk and postnatal maternal hair will be collected. Measurements will include environmental chemicals, nutrients, nutritional status markers, biomarkers of genetic polymorphisms, immunoprotective constituents, and biomarkers of smoking. The questionnaires include prenatal questionnaires during the 1st and 3rd trimester, chart review questionnaires during 1st, 2nd, 3rd and at delivery, a food frequency and dietary supplement questionnaire, a postpartum questionnaire and a lactational questionnaire (see Appendix 2).

All biospecimen containers (including those specially pre-cleaned for the POPs analyses) will be provided by the CTQ, INSPQ laboratory to ensure conformity in batches of supplies. Figure 2 summarizes the collection phases for the study. All questionnaires (case report forms) and biospecimen collection containers will only be identified by a unique identifier or bar code for each woman. Only the clinic site will have the key to link these unique identifier numbers with the names and contact information for each participant.

Figure 2. Overview of measures taken at each collection phase



13. Prenatal and Perinatal Biomarkers of Heavy Metal Exposure, Nutritional Status, and Genetic Susceptibility

a) Specimen and data collection

Maternal specimens will be collected at the time of prenatal clinical contacts and at delivery (see Figure 2). Blood and urine samples will be collected during the 1st and 3rd trimester and at delivery for heavy metals, selenium, bone turnover and oxidative stress markers. Analysis of maternal samples for other chemicals (phthalates, bisphenol A, persistent organic pollutants [brominated flame retardants, organochlorine pesticides, polychlorinated biphenols], organophospate pesticides, perfluorinated compounds, and cotinine) will depend on availability of funds. Meconium will be collected and pooled for each infant over the first 2 days post-natally. Meconium (the first several stools passed by a newborn after birth) begins to form *in utero* around the 13th week of gestation and accumulates thereafter and may provide a longer and cumulative record of exposure to various environmental chemicals than urine or cord blood (Bearer 2003). Meconium is composed of amniotic fluid, mucous, lanugo (the fine hair that covers the baby's body), bile, and cells that have been shed from the skin and the intestinal tract (Medline Plus http://www.nlm.nih.gov/medlineplus/ency/article/002262.htm). Meconium is thick, tar-like, greenish black, and sticky. As meconium is easily obtained and noninvasive, it is an ideal matrix for measuring *in utero* body burdens of contaminants. Lead, mercury and cadmium, have been detected to varying degrees in the meconium of neonates (Ostrea et al., 2002). Prenatal and post-delivery questionnaires will be administered to collect information on potential confounders of the relationship between exposure to chemicals and adverse pregnancy outcomes, as well as to identify characteristics of women with higher and lower exposure to heavy metals.

b) Measurements required and laboratory support

- whole blood heavy metal concentrations (lead, mercury, cadmium, arsenic, manganese) in 1st (baseline) and 3rd trimester (using trace metal free vacutainers); 5 mL required
- selenium and glutathione peroxidase activity in plasma during 1st and 3rd trimesters
- vitamin D [25(OH-D)], parathyroid hormone (PTH), and osteocalcin (bone formation) in maternal serum at 1st, 3rd and 4th visit and in cord blood, and telopeptide (bone resorption) in maternal urine at 1st, 3rd, 4th visit and cord blood
- biomarkers of oxidative stress in first and third trimester and at birth from plasma (3 mL) and urine samples (5 mL)
- markers of bone turnover (pyridinium compounds) in spot urine (corrected for creatinine) collected during 1st and 3rd trimesters (2 mL)
- endothelins in third trimester and at birth in plasma (3 mL)
- metals in meconium (lead, mercury, cadmium, arsenic, manganese) (at least 0.2 g wet meconium)
- maternal genotyping at *Plateforme de séquençage et de génotypage des génomes* for a number of SNPs potentially associated with susceptibility to metal and other environmental chemical toxicity or adverse pregnancy outcomes of interest
- prenatal questionnaires administered in the 1st and 3rd trimester will collect data on potential sources of exposure to these contaminants and on other important covariates
- a food frequency and dietary supplement questionnaire will be administered during pregnancy to obtain data on the woman's nutritional status (especially calcium, iron and anti-oxidants).

All biological specimens will be stored and then shipped in batches to the laboratories for analyses. The Centre de toxicologie laboratory is accredited by the Standards Council of Canada under ISO 17025, the international standard for technical competence and quality in all areas of testing and calibration. Biological specimen handling and shipping instructions are given in Appendices 12 and 13. Heavy metals (i.e., lead, mercury, cadmium, manganese, arsenic) are measured using inductively-coupled plasma-mass spectrometry, the present state-of-the-art technique for measuring metals (Centre-de-toxicologie-du-Québec. May 2005). It affords high sensitivity, specificity and accuracy and enables the simultaneous determination of several metals in the same sub-sample. Detection limits are: Blood Pb (0.001 µmol/L), Cd (0.4 nmol/L), total Hg (0.5 nmol/L), Mn (1 nmol/L), As (3 nmol/L).

Oxidative stress and vasoregulatory markers will be analysed in the Health Canada laboratory of Drs. Vincent and Kumarathasan who have extensive experience in this field (Kumarathasan, Goegan et al. 2001; Kumarathasan and Vincent 2003). The oxidative stress markers that will be analysed are plasma isoprostane by an EIA assay and plasma tyrosines (p-,m-,o-,Cl-, 3-nitro), uric acid, L-DOPA, 8-hydroxydeoxyguanosine and also norepinephrine and epinephrine by HPLC-coulometry array method. As for vasoregulatory components, we will measure circulating plasma big-ET1, big-ET2, big-ET3 and corresponding mature peptides ET1, ET2 and ET3 by the HPLC-fluorescence method. Free nitrite levels in plasma will be analysed by a fluorescence method. The costs of these analyses will be covered by Health Canada.

Plasma and urine biomarkers of nutritional status and bone resorption will be measured at the Health Canada laboratories of Drs. Cockell and Hidiroglou. Urine markers of bone turnover (pyridinium compounds) will be measured on samples collected at 1st and 3rd trimester visits to assess the degree of change, as the majority of Ca transfer to the fetus occurs during the 3rd trimester (IOM 1997). Commercially available EIA kits will be used (e.g. Metra PYD EIA kit, Quidel Corp. San Diego, CA, or NTx Reagent Pack, Ortho-Clinical Diagnostics, Rochester, NY). (Ionized calcium levels can not be measured due to difficulties with collecting and storing serum samples for ionized calcium levels in a multi-center study: http://www.labcorp.com/datasets/labcorp/html/chapter/mono/sr001100.htm).

Serum glutathione peroxidase activity (antioxidant enzyme and index of selenium status, IOM 2000) will be measured in samples taken at 1st and 3rd trimesters (L'Abbe, Fischer et al. 1989) with costs covered by Health Canada. Vitamin D status will be assessed using serum 25(OH)D and PTH at recruitment, late pregnancy (32 to 34 wk) and at delivery in the mother and in cord blood to provide for maternal-fetal transfer of 25(OH)D and fetal PTH response. Bone formation will be examined in blood samples for mothers at 6-13 and 32-34 wk, delivery and cord blood. Bone resorption will be measured in spot urine samples in pregnant women at 6-13 and 32-34 wk, delivery if feasible and an assessment in infants will be measured in cord blood. Vitamin D status will be measured using a RIA (serum/plasma; 50-100 µl) and serum intact PTH will be measured using an ELISA (50-100 µl). Change in bone metabolism in response to 25(OH)D will be assessed by a marker of osteoblast activity related to mineralization, plasma osteocalcin (25-50 µl). Osteoclast activity will be assessed by measuring serum/urinary C-telopeptide or N-telopeptide (25-50 µl ELISA) corrected to creatinine. A deficiency of vitamin D in pregnant women will be taken as ≤37.5 nmol/L and for newborn infants at ≤27.5 nmol/L. Optimal vitamin D status will be defined as a serum 25(OH)D value of ≥75 nmol/L. This concentration is derived from dose-response studies of the relationship between 25(OH)D and PTH, where PTH plateaus in the mid-normal range and also from studies where 25(OH)D concentrations of 90 to 100 nmol/L are positively related to bone mineral density in young adults. Optimal 25(OH)D concentration for infants is unknown but is projected to be at least 75 nmol/L based on adult studies.

Polymerase chain reaction (PCR) restricted fragment length polymorphism (PCR-RFLP) will be used for genotyping of gene polymorphisms of interest for metal toxicity/susceptibility for mothers. DNA will be extracted from whole blood samples and analyzed at Geno-Quebec.

14. Prenatal and Perinatal Smoking Habits and Exposure to Other Priority Environmental Chemicals

a) Specimen and data collection

The *questionnaire*-based approach will prospectively collect data on *smoking* behaviour, passive smoking, cessation of smoking and relapse at each of the following study visits (6-13 weeks, 32-34 weeks of gestation, and after delivery) as well as information on occupation, hobbies and the home which may be potential sources of exposure to the contaminants measured. The biological marker-based approach will assay levels of creatinine and specific gravity, bisphenol A, speciated arsenic, organophosphate pesticides and phthalates in maternal urine, serum cotinine, plasma brominated flame retardants, perfluorinated compounds, PCBs, OCs as well as nicotine and cotinine in meconium samples of the newborns.

Blood specimens will be collected at the time of clinical contacts during each trimester and at delivery. Urine samples will similarly be collected at each prenatal visits and at delivery, stored and then shipped in batches along with the blood samples to the laboratory for chemical analyses, Centre de Toxicologie du Québec, Institut national de santé publique du Québec, 945, avenue Wolfe, Sainte-Foy, PQ). Biological specimen handling and shipping instructions are given in Appendix 6. Biospecimens will be processed and stored in the central biorepository laboratory at Ste. Justine Hospital and then shipped to the CTQ/INSPQ laboratory for analyses on an intermittent basis depending on funding availability throughout the 5-year period of the study.

Maternal blood and urine samples, umbilical cord blood, and infant meconium will be collected using the appropriate containers during pregnancy to estimate maternal and fetal exposure to environmental chemicals. All samples will be stored and then shipped in batches to the laboratory for analysis for brominated flame retardants, other persistent organochlorine chemicals, organophosphate pesticides, phthalates, bisphenol A, and smoking by-products.

b) Measurements required and laboratory support

- maternal serum for cotinine and pefluorinated compounds;
  maternal plasma for PBDEs, PCBs and other persistent organic pollutants; 5ml
- maternal urine for phthalates, bisphenol A, speciated arsenic, organophosphate pesticides, specific gravity and creatinine; 120ml
- cord blood for heavy metals and other environmental chemicals;
- meconium for nicotine and cotinine, heavy metals and other environmental chemicals;
- prenatal and post-delivery questionnaires to identify characteristics of women with higher and lower exposure to priority chemicals other than heavy metals.

High performance liquid chromatography coupled to a tandem mass spectrometer (HPLC-MS-MS) will be used for the measurement of cotinine/nicotine. The techniques are extremely sensitive and specific.

15. Lactational Exposure: Nutritional, Environmental and Immunoprotective Analytes in Human Milk and Analysis of Maternal Hair for Mercury

a) Specimen and data collection

The human milk component will be conducted on a sub-set of the cohort who will be breastfeeding. According to the report on perinatal health indicators for Canada (Health-Canada 2000), the national breastfeeding rate is estimated at 73% with some variability between Provinces. By overestimating to 75%, it is expected that approximately 1500 of the 2000 will be eligible to participate. Human milk will be collected for measurements of nutrients, environmental chemicals and immunoprotective constituents as well as a sample of hair for measurements of total mercury (see Table 2 for complete listing of the analytes). A questionnaire will be used to obtain dietary, breastfeeding pattern and limited but relevant health related information. We seek to achieve a greater understanding of the potential sources which may influence the concentration of the analytes in the milk. The questionnaire will take approximately 15 minutes to complete.

Research nurses at each site will be trained by Health Canada staff and provided with the appropriate number of kits. The kit, available in French and English, includes: a questionnaire, pre-cleaned wide-necked jars in which to express the milk, clear instructions for collecting and storing the milk (Appendix 8), clear instructions for completing the questionnaire, and instructions to assist with expression of milk. No personal information will feature on the questionnaire or sample containers. The numerical code, which would have been already assigned to the participant, will be printed on the questionnaire and sample containers. The nurse will follow-up with the mother by phone two weeks following the first postnatal visit to ensure that the mother clearly understands the instructions and to answer any potential concerns.

Mothers are asked to donate a cumulative volume of 200 ml (approximately ¾ cup) of mature milk (3-8 weeks postpartum) after or while the infant is nursing on the other breast, to take advantage of the let-down reflex and to obtain an optimum combination of fore and hind milk. She will also be asked to provide milk from several feedings throughout the day or over several days if necessary. Variation in the fat content does occur during lactogenesis and during any particular feeding period (Needham and Wang 2002). During the first few days post-partum, the fat content in colostrum is approximately 2.9%. After 2 weeks, colostrum becomes mature milk with its fat content stabilizing at approximately 4% (Needham and Wang 2002). Further, at any particular feeding, fore milk which is produced at the beginning of the feed, contains many nutrients but is low in fat where as hind milk which is formed later in the feed, contains many nutrients but is rich in lipids. Fat content of an optimum combination of fore and hind mature milk is desired (Mes 1994). Mothers will be asked to express their milk manually to reduce potential contamination from the use of breast pumps. It is stated in the instructions given to the mother and communicated by the research nurses that manual expression of milk is preferred, but breast pumps may be used if the mother is having difficulty manually expressing milk. The mother will be provided with an illustrated visual pamphlet to assist with manual expression or for additional help, she may contact the nurse who gave her the kit.

Mothers will be provided with two pre-cleaned wide-necked (one amber glass and one plastic to accommodate the different types of analyses) jars for the milk sample and clear instructions on how to collect and store the milk. The portions collected during each feeding should be added to the collecting bottle and stored in the home refrigerator until the required volume has been expressed. A line on the collection jar will mark the minimum amount required. The milk may be stored in the refrigerator at 4^{o}C for a maximum of 72 hours. For longer times, the milk should be

stored in the home freezer. The protocol for the collection and handling of human milk is adopted from the human milk survey protocol established by the World Health Organization (WHO-(World-Health-Organization) 2004).

The participant is asked to answer the questionnaire after completion of the milk collection. Once the mother has completed the questionnaire and expressed the required amount of milk, she will be asked to phone and inform the nurse. The research nurse will then perform a visit to the mother's home to collect the package. During this visit, a sample of the participant's hair will be cut by the trained nurse. For those participants who have declined participation in the human milk survey, hair samples will be collected at visit 5 (ie. 1-2 days postpartum). The hair collection procedure for mercury analysis will be performed as follows. A 5 mm (thickness of a pencil) bundle of hair will be isolated and cut from the occipital region, ensuring minimum damage to the participants' aesthetics. The hair will be placed in a polyethylene bag and fastened with two staples near the scalp end. The nurse will also administer a 10-minute food frequency questionnaire similar to the one administered prenatally.

The nurse will ensure that the completed (or uncompleted) questionnaire, the milk and hair samples are well-packed. The packaging will follow the requirements of the International Air and Transport Association (IATA) for diagnostic specimens UN3373 (packaging: TC-125-1B). The questionnaires are sent to the Ste. Justine's Hospital Coordinating Center for data entry. Hair and milk samples are sent back to the Food Research Division in Ottawa by prepaid shipment. Upon receipt of the kits at the Food Research Division in Ottawa, each milk sample will be partitioned into smaller volumes to provide each laboratory with the amount of milk necessary to perform the planned analysis. Each participating laboratory is equipped with -20^{o}C and -80 ^{o}C freezers such that samples are appropriately stored until analysis is performed. For the measurement of most analytes, the samples can be stored indefinitely at -80 ^{o}C. See Table 2 for instrumentation used to perform the analyses. No special storage considerations are required for the hair samples.

b) Measurements required and laboratory support

The human milk component will include those chemicals that will be analyzed in the prenatal component, except for arsenic and those that have been analyzed in past Health Canada human milk surveys (which correspond to the chemicals outlined in the Stockholm Convention). Additionally, chemicals of more recent concern, and, for the first time, inclusion of certain nutrients and immunoprotective constituents will be included in the analysis. All measurements will be performed by Health Canada laboratories. Table 2 lists all the analytes and below is the rationale for their selection. The laboratory methodology employed for the measurements of the analytes in human milk and hair are recognized worldwide and are extremely sensitive and specific.

Table 2. Human milk volume required to perform analysis of the various analytes, instrumentation used in the laboratory and the researcher responsible for the analysis.

Analyte (s)	Amount required (ml)	Instrumentation	Laboratory responsible for analysis
Heavy metals -Cadmium, lead -Mercury	25 shared[b]	Inductively coupled plasma – mass spectrometry CETAC M-6000 mercury analyzer	Dr. Robert Dabeka

Fungal toxins - Ochratoxin A and its metabolite ochratoxin alpha	10	Liquid chromatography – fluorescence detection	Gary Lombaert and Veronica Roscoe
Organohalogens *Pesticides* -Dichloro-diphenyl-trichloroethane and metabolites, dieldrin, heptachlor, oxychlordane, hexachlorocyclohexane, transnanochlor [aldrin, mirex][a]	50 shared[b]	Gas chromatography – mass spectrometry	Dr. Thea Rawn
Organohalogens *Industrial products* - Hexachlorobenzene, polychlorinated biphenyl congeners, polybrominated diphenyl ether congeners	50 shared[b]	Gas chromatography – mass spectrometry	Drs. Jake Ryan and Thea Rawn
- Perflurorinated organics, hexabromocyclododecane, [decabromodiphenylethane, 2,4,6-tribromomethoxyethane][a]	20	Gas chromatography – mass spectrometry Liquid chromatography – mass spectrometry – mass spectrometry	Dr. Sheryl Tittlemier
Organohalogens *Industrial by-products* - dioxins and furans	50 shared[b]	Gas chromatography – mass spectrometry	Drs. Jake Ryan and Thea Rawn
Other contaminants of concern - Phthalates	5	Gas chromatography – mass spectrometry	Dr. Xu-liang Cao
- Perchlorate	5	Ion chromatography – mass spectrometry	Dr. Don Forsyth
- bisphenol A	10	Gas chromatography – mass spectrometry	Dr. Xu-liang Cao
- MCPD esters	5	Gas chromatography – mass spectrometry	Dr. Adam Becalski
Trace elements			

- Calcium, magnesium, phosphorus, potassium, and sodium - Aluminum, barium, beryllium, chromium, copper, iron, lithium, manganese, molybdenum, thallium, uranium, vanadium, and zinc [selenium, and iodine][a]	10 25 shared[b]	- Atomic absorption (Ca, Mg, Na, K) - Colourimetric analysis (P) Inductively coupled plasma – mass spectrometry	Dr. Kevin Cockell Dr. Robert Dabeka
Vitamins -Vitamin D, E, and carotanoids -Folic acid and total folates	17 20 shared[b]	High performance liquid chromatography (HPLC) HPLC and microplate, microbiological analysis	Dr. Nick Hidiroglou Dr. Nimal Ratnayake
Macronutrients -Fatty acid profile	20 shared[b]	Gas chromatography	Dr. Nimal Ratnayake
Endogenous bioactive constituents - Secretory IgA - Lactoferrin, - Lysozyme, - Prolactin - Cytokine IL-6 - total antioxidant capacity, glutathione peroxidase and superoxide dismutase activity - gamma-glutamyl transferase, myeloperoxidase, lactoperoxidase, lipid peroxides, and oxidized LDL	5 8	96-well platereader to measure enzyme and ELISA endpoints	Dr. Genevieve Bondy Dr. Dawn Jin
Storage	10	To be decided	To be decided
Maternal Hair total mercury	5 mm hair bundle	Mercury analyzer	Dr. Melissa Legrand

a. The methodologies used to determine the concentration of these analytes are currently being developed.
b. "Shared" refers to the volume of milk that will be shared between laboratories to undertake the various analyses. For example: to perform the folic acid/total folates and the fatty acid profile analysis, 20 ml will be shared.

Rationale for selection of analytes for the human milk survey

For organic contaminants, the analytes will include the majority of the chemicals that have been analyzed in past surveys which coincide with the 12 priority POPs outlined in the Stockholm convention, except for toxaphene. Other industrial POPs of more recent concern and with widespread occurrence and demonstrated toxicity (Gill, Chu et al. 2004; Kubwabo, Stewart et al. 2005) that will be analysed as part of the survey include polybrominated diphenyl ethers, perfluorinated organics, phthalates, perchlorate, hexabromocyclododecane, decabromodiphenylethane, and 2,4,6-tribromomethoxyethane. At present, it is uncertain whether the presence of some of these analytes impacts on human health (Ryan and Patry 2001). In order to characterize the risk from these environmental chemicals, data on human exposure is required. Analysis of human milk for these environmental chemicals provides the means to establish baseline levels and supply the relevant information for risk assessment.

Heavy metals such as lead, cadmium and mercury remain priority chemicals and will also be featured in the human survey. Other metals will include: aluminum, barium, beryllium, cesium, lithium, rubidium, strontium, and thallium. Because of their preferential solubility in aqueous solution, they appear in milk at smaller concentrations than lipid soluble chemicals like POPs. They are about 20% of the level found in blood from the same person (Boischio and Henshel 2000; Needham and Wang 2002). The heavy metals cadmium and lead were measured in a previous survey, that of 1982. The levels were within a safe range, and were correlated to environmental and dietary sources of exposure (Dabeka, Karpinski et al. 1986). The results from this survey will serve to establish baseline data for mercury, aluminum, barium, beryllium, cesium, lithium, rubidium, strontium, and thallium, to allow time-trend analysis for lead and cadmium and to re-evaluate the associations between the dietary and environmental attributes which were previously correlated with the concentration in the milk.

One food processing induced chemical will be measured in milk. 3-Chloropropane-1,3-diol is currently considered a non-genotoxic carcinogen with a provisional tolerable daily intake (pTDI) of 1.1 ug/kg bw/day as calculated by Health Canada's Bureau of Chemical Safety. Exposure to 3-MCPD esters is recognized to be the major potential source of 3-MCPD, (Codex Alimentarius Commission, CX/CF 07/1/13, Feb. 2007). The occurrence of 3-MCPD esters at an average of 36 ng/g (n=12, range 11-76) was also shown in human milk in the Czech Republic. (Zelinkova et al. Food Addit. Contam. 25, 2008 669-676). These 3-MCPD esters are likely to occur in breast milk due to mothers' dietary exposure. The authors calculated that JECFA's (2001) TDI of 2 ug/kg bw/day would be exceeded by a factor of 4 assuming a total hydrolysis of 3-MCPD ester. Our laboratory has recently shown that 2-MCPD esters are also present in come commercial fats at the levels approaching 50% of 3- chloro isomer. It is plausible that 2-MCPD esters also bioaccumulate and can be excreted in mother's milk. Toxicological significance of 2-MCPD is unknown due to the lack of data. However, a precautionary principle warrants interest in the presence of 2-MCPD in foods. The data from MIREC regarding the presence of 2-MCPD would be the first of its kind.

The survey will also include analysis of Ochratoxin A, a common storage mould in temperate areas such as Canada (Scott, Kanhere et al. 1998). Ochratoxin A has been found in foods of plant origin, and in the edible tissues of animals, particularly pork. The major target organ for ochratoxin A in all mammalian species is the kidney (Scott 2005). Its presence in Bulgarian foodstuffs has been associated with the kidney disease endemic to that area (Petkova-Bocharova and Castegnaro 1991). Recent studies have provided clear evidence for the carcinogenicity of ochratoxin A in two rodent models and it has been found to be a potent renal carcinogen in rats (Kuiper-Goodman and Grant 1991). Ochratoxin A has been found in human blood sera in Europe and in Canada (Scott

2005), and in human milk in Sweden (Breitholtz-Emanuelsson, Olsen et al. 1993), Switzerland (Zimmerli and Dick 1995) and Germany (Bauer and Gareis 1987). Nursing infants may be particularly susceptible to exposure from mother's milk because of the high milk intake relative to their body weight, and because of the limited variety of their diet. A survey of the incidence and levels of ochratoxin A in Canadian human milk has not been undertaken, thus a knowledge gap. Such data would be valuable to determine the exposure of infants to this toxin.

This survey is not only limited to assessment of contaminants. The Bureau of Nutritional Sciences will be undertaking analysis of a suite of mineral nutrients (calcium, magnesium, phosphorus, potassium, sodium, chromium, copper, iron, manganese, molybdenum, vanadium, and zinc), several vitamins (folate, vitamin D, vitamin E and carotenoids) and a complete fatty acid profile (including trans-fatty acids and omega-3). Given that human milk is the predominant and often the sole-source of food for infants, reference standards for definition of infant nutritional needs are based on levels in human milk. The national scope of the study provides superior "baseline" data to existing published data which are limited in numbers of subjects and/or geographic range. These data will fill a knowledge gap regarding nutrient composition of human milk across Canada.

In addition to providing vital nutrients to infants, milk also provides a number of bioactive factors that are vital for protecting newborns in the early postnatal period when the mucosal and systemic immune systems are still developing (Kelleher and Lonnerdal 2001). These endogenous constituents include immunomodulatory, anti-microbial and anti-inflammatory agents that are protective for both the newborn and for the mammary gland itself. Human milk samples will be analysed for secretory IgA, lactoferrin, lysozyme, prolactin and the cytokine IL-6 using an enzyme-linked immunosorbent assay (ELISA) for each constituent.

A number of antioxidants and antioxidant enzymes have been found in human milk, which include alpha-tocopherol, cysteine, glutathione, ascorbate, carotenoids, selenium, glutathione peroxidase, superoxide dismutase, and catalase, although a complete list of active antioxidants present in human milk is yet to be established. Lipids, especially n-3 polyunsaturated fatty acids are important components of human milk, which are critical for infant's brain development. However, in the presence of heavy metals and other contaminants, essential fatty acids may be oxidized, and thus lose their nutritional value or even become harmful to infants. Heavy metals such as Pb, Cd, and Hg and other contaminants are known to exert toxicity through increased oxidative stress. Thus the potential correlation between maternal body burden of contaminants and oxidative and antioxidant status of human milk will also be examined. This will assist the risk/benefit assessment for human milk. Antioxidant status will be determined by measuring total antioxidant capacity, glutathione peroxidase and superoxide dismutase activity, while oxidative status will be assessed by measuring gamma-glutamyl transferase, myeloperoxidase, lactoperoxidase, lipid peroxides, and oxidized LDL.

Maternal Hair Analysis for Mercury

In addition, mercury will be determined in maternal scalp hair samples. The hair-Hg results will be used to corroborate mercury levels measured in breast milk. Further, with a hair growth rate of approximately 1-cm per month (Robbins 2002), fetal exposure during gestation can be assessed with determination of Hg in maternal hair samples (Cernichiari, Brewer et al. 1995). Hair samples are easily obtained and the collection is non-invasive which makes it a practical biomonitoring tool in large populations (Cox, Breazna et al. 1999; Grandjean, Budtz-Jorgensen et al. 1999). It is important to note that mercury remains a public health concern and the need for biomonitoring of hair-Hg levels in this population has been raised and identified as a knowledge gap.

16. Storage of Residual Biospecimens

MIREC will collect blood and urine from approximately 2000 pregnant women at several critical time periods during pregnancy and at delivery as well as cord blood and infant meconium. Of the 2000 women, human milk samples will also be collected from eligible and willing participants.

The proposal is to collect the same volume from each participant and to perform laboratory analyses using the following strategy which reflects funds currently committed. For the samples collected prenatally and infant meconium, heavy metals analyses, oxidative stress and bone turnover biomarkers will be performed for each individual but determination of expensive but valuable POPs analyses will be undertaken as funds are available. Similarly, all breast milk samples will be analysed for heavy metals, ochratoxin A, phthalates, trace minerals and immunoprotective constituents. A sub-sample of milk samples representative of the study population will be analysed for the remaining analytes listed in Table 2.

We propose to store residual samples for the following reasons:

- As additional funds become available, the stored samples will enable future analyses of all proposed analytes in each sample which will serve to complete the profile of each participant. Selection of analyses, as mentioned above, was based on current committed funds. This is closely related to the fact that while affirmation for long term participation in this initiative has been obtained from senior management of HECS and FD, financial commitment from Health Canada may only be secured one year at a time. This being said, we are confident that moneys will become available to perform the remaining analyses.
- The stored samples will also enable future analyses of limited but emerging chemicals of concern as analytical methods become developed and validated. The relative fast pace of technological advancements, especially in laboratory methodology; will permit detection of potentially toxic, man-made substances which have thus far remained undetected. The case of brominated flame retardants (PBDEs) illustrates this point. Although PBDEs have been in use for several decades (Solomon and Weiss, 2002), only recently have laboratory methods been capable of routine measurements. Through the use of stored human milk samples, retrospective analyses revealed a significant increasing trend of PBDEs in Canadian samples over a decade (1992-2001) (Ryan and Patry 2001). This and other similar data demonstrated its widespread occurrence in the populace and biota and were used to successfully support a ban of PBDEs in European markets.
- The cost associated with recruitment of a representative sample of these rare yet most susceptible and vulnerable populations is quite appreciable as demonstrated in the proposed budget. In this context, it may be considered prudent to build on this infrastructure and maximize the return of important investments of time (especially that of the mothers), resources, and public funds.

The participants will be informed in the consent form of the planned analyses and the duration of storage. A separate informed consent form will be used to ask permission to store the participant's biological samples for future but as yet unspecified analysis as part of the MIREC study. At any time, the participant may request that her sample be destroyed regardless of the time passed since collection took place. Confidentiality is ensured by printing only the numerical codes on the sample containers. The samples will be stored in secure wings of Health Canada and Ste-Justine Hospital with access to freezers being restricted to the research team. Biospecimens will be stored for a maximum of 30 years then destroyed as follows: the labels on the specimens

vials will be blanked out by marker pen and the vials will be sent through the normal waste stream including autoclave of the vials and disposal. The biospecimens in the biobank will continue to contain the unique identifying bar-code until the project is completed or the 30 years has passed. This will facilitate being able to destroy the biospecimens of anyone wishing to withdraw from the study and also allow the linkage of the specimen with the data collected on the health and co-factors for that individual. Only the site investigators and the coordinating center will have access to the key to link the unique bar-code to the names and contact information for the participant.

17. Pregnancy Outcome Assessment

The primary health outcomes to be evaluated are elevated maternal blood pressure and fetal growth retardation (< 10th percentile of birth weight by gestational age) (Kramer, Platt et al. 2001). Preterm delivery and sex ratio at birth will also be evaluated.

The outcome measures of maternal blood pressure during each clinic visit and infant birth weight and gestational age are collected according to specified criteria. Systolic and diastolic blood pressure will be measured by the clinical staff at each visit using a sphygmomanometer. Blood pressure is assessed in a sitting position, with the cuffed arm resting on a desk at the level of the heart. Diastolic blood pressure should be measured at Korotkoff phase V (disappearance), and IV (muffling) only utilized when a phase V is absent. Gestational hypertension will be defined as two (2) or more readings of diastolic blood pressure greater than 90 mmHg taken 4 hours apart and occurring after 20 weeks of pregnancy. *Severe gestational hypertension* will be defined as two recordings of diastolic blood pressure of ≥110 mmHg or systolic pressure ≥160 mmHg at least 4 hours apart.

In order to facilitate follow-up and to obtain all test results and indicators to assess the primary outcome, the recruited patient's medical files will be labelled with a special trial mark. If prenatal charts are not included in the hospital record, the clinical staff must contact the office of the treating physician to obtain a signed and dated copy of the prenatal charts. Data on delivery and neonatal status will be abstracted from mother's and baby's hospital charts as soon as possible after hospital discharge whereas the data of the pregnancy would be captured through out the perinatal follow-up. If prenatal charts are not included in the hospital record, the research assistant must contact the office of the treating physician to obtain a signed and dated copy of the prenatal charts. For patients who deliver in a centre other than the one initially planned, the research assistant should contact the medical center and obtain a signed and dated copy of the delivery chart.

18. Other Covariates

Other covariates such as nutritional status in early and late pregnancy, lifestyle and demographic factors will be assessed from the questionnaires and clinical records.

19. Reporting of Individual Environmental Chemical Analysis Results

Each participant will be provided with their individual results for the heavy metal analyses (maternal blood lead, cadmium and mercury) through their doctor or health care provider if they are higher than health-based guidelines that are scientifically proven to be significant for the health of the participant and there are preventive measures or treatments available. If this is the case, the study team will endeavour to have the heavy metal results available to the participant within three months of collection. A Results Report Advisory Committee was convened by Health Canada in November 2007 to identify those chemicals for which there are health-based tissue

guidelines and to suggest the kind of material for each environmental chemical that should be included in the information package for the health care providers. This material will include the most up-to-date information on potential sources of exposure, the range of levels measured in other population surveys, levels of concern, potential health effects, and approaches to reducing exposure. Once this package of material was developed, known as Physician Guidance Documents for Lead, Cadmium and Mercury, and associated Physician letters, it was submitted to the various ethics committees for approval, starting with Health Canada's REB.

In Quebec lead, mercury, cadmium, manganese and organophosphate pesticides fall under Reportable Diseases [maladies et intoxications à déclaration obligatoire (MADO)]. Therefore our Montreal site at Ste Justine and the Jewish Hospital in Montreal are legally obliged to report elevated results to the public health department, according to the MADO guidelines. Physician letters were developed for the Montreal site to communicate any maternal blood results that exceed the Quebec reportable levels (See Appendix 11). For cadmium, both MIREC and MADO levels are 5.1 ug/L; for mercury, the MIREC guideline is 8 ug/L, whereas, the MADO level is 12 ug/L; for lead, the MADO value is 10 ug/dL, whereas MIREC uses 4 ug/dL. There are no health-based guidance values for manganese or organophosphate pesticides in MIREC as our results report experts felt that especially in pregnancy, a blood manganese level was "uninterpretable" and the evidence was insufficient to develop guidance values for the organophosphate pesticides.

Environmental Chemical	Reportable Level (MIREC Study)	Reportable Level (Quebec)
Lead	4.0 µg/dL (0.19 µmol/L)	10.0 µg/dL (0.5 µmol/L)
Mercury	8.0 µg/L (40 nmol/L)	12 µg/L (60 nmol/L)
Cadmium	5.1 µg/L (45 nmol/L)	5.1 µg/L (45 nmol/L)
Manganese	N/A	365 nmol/L
Dimethylphosphate	N/A	65 µg/L
Dimethylthiophosphate	N/A	230 µg/L
Dimethyldithiophosphate	N/A	95 µg/L
Diethylphosphate	N/A	65 µg/L
Diethylthiophosphate	N/A	10 µg/L
Diethyldithiophosphate	N/A	5 µg/L

20. Training

A one-day training session was held in Montreal in February 2007 for all site investigators and research nurses to describe the objectives of the research study, eligibility criteria, and the protocol and train the research staff on recruitment, consent, administering questionnaires and collecting, processing and shipping the biological specimens. A further one-day training session will be held in 2007 (if necessary) to train the research staff on the final version of the protocol that has been approved by Health Canada's and Ste. Justine's Hospital ethics boards. A training manual will be provided to the research staff along with the appropriate contact information. The manual will include step-by-step instructions for each collection phase, including illustrated instructions for the collection of maternal hair and infant meconium. Annual meetings of the site investigators will be held each year to discuss the study progress and preliminary results. In addition, during the first 12-months of participant recruitment, the research coordinator will visit each site twice and annually during subsequent years to audit the participant recruitment, consent, and data and sample collection processes to ensure that each site is adhering to the study protocol.

21. Data Management and Statistical Analyses

The prenatal questionnaires (the 1st, 2nd and 3rd trimester) and the lactational questionnaire will be administered by the research nurse. One copy will be retained at the clinic site and the original will be sent in batches to Ste. Justine's Hospital where the data will be captured using computer database software. The data will be stored on a secure password protected server at Ste. Justine's Hospital. No personal identifying information will be included on the questionnaires or the sample containers. Each questionnaire and biospecimen container will contain a unique bar-coded identifier. Only the clinic site and the Study Coordinating Center will have the key to link the unique identifier with the names and contact information for each participant.

Descriptive statistics will be used to describe the patterns of heavy metal exposure (as well as other priority environmental contaminants) for the mother and infant throughout the pregnancy. The primary health endpoints to be measured in this study are maternal blood pressure and fetal growth retardation. It is expected that the heavy metal data will be natural log-transformed to normalize the variables for statistical procedures. Multiple regression analysis appropriate to the endpoint will be used to assess the risks (e.g., multiple regression model with blood pressure as the dependent variable to determine whether maternal blood lead (BPb) was independently associated with variations in blood pressure; dichotomous indicator of fetal growth retardation in a logistic regression analysis to determine the odds ratio for meconium cadmium concentration adjusting for various potential confounders such as body mass index, age, parity, smoking and educational level). A covariate will be considered to be a confounder of the relation between body burden of the heavy metal and the adverse pregnancy measure if the variable is an independent risk factor for the disease and if adding the variable to the multivariable model changes the point estimate by 10% or more. To test for effect modification (e.g., selenium levels with maternal body burden of lead), interaction variables will be created and entered into the model. Adjusted logistic regression analysis will be used to test whether genetic polymorphisms are associated with increased odds for elevated blood pressure or fetal growth retardation (e.g., interaction term of ALAD allele status x log BPb concentration). Likelihood ratio tests will be used to assess the significance of the interaction term.

Scaled linear mixed models will also be used to assess the effects of exposure and other covariates on the multiple continuous outcomes of maternal blood pressure and fetal growth (Lin, Ryan et al. 2000). Correlations among different outcomes can be accommodated by using random effects using SAS PROC MIXED.

Many of the earlier studies of heavy metals only measured one contaminant. Measurement of other metals might account for additional variance and thereby increase the power to detect an effect for one contaminant (Rice 2005). As human chemical exposures are rarely to individual agents but to mixtures, and all chemical exposures occur in a context with other risk factors such as genetic background, dietary status, lifestyle, and socioeconomic status, this collectively yields a complex set of interactions with the ultimate expression manifested in some degree of health effect (Cory-Slechta 2005). Complex statistical analyses are required to assist in identifying effect modifiers and to model the exposure (body burden) - effect function.

22. Power Calculations

With a sample of 1800 women, for the dichotomous outcomes (gestational hypertension or IUGR) with a baseline incidence in the study population of 5% and comparing the highest quintile of heavy metal concentration with the lowest (e.g., ≥ 4 versus ≤ 1 µg/dl for lead), the power is 89% to detect a risk factor with a relative risk (RR) of 2 (alpha = .05). For the same population size and

blood pressure or birth weight as continuous variables, the power was 91% to detect any risk factor with an effect size of 0.2 SD difference. We will recruit 2,000 women to allow for some loss to follow-up and missed specimens. Should additional funds become available, we will increase the sample size to 3,000 women, which will provide more statistical confidence in the risk estimates, especially when interactions between chemicals (mixtures) are considered.

The inconsistencies observed among studies of pregnant women suggest that either the sample size was too small (fewer than 700 women) or one measurement of maternal blood lead may not be a good indicator of exposure during pregnancy to adequately reflect endogenous exposure from bone lead mobilization (Sanin, Gonzalez-Cossio et al. 2001). There has been some discussion about the appropriate metric of lead exposure to predict fetal toxicity (Chuang, Schwartz et al. 2001), with bone lead generally accepted to be ideal (but impractical for a multi-center study) and while plasma measures biologically available lead levels, it can be very challenging to collect because of fears about contamination during collection, manipulation (e.g., plasma hemolysis) or storage (Smith, Hernandez-Avila et al. 2002; Barbosa, Tanus-Santos et al. 2005). We propose to collect maternal 1st and 3rd trimester blood, cord blood and meconium samples for heavy metal analyses. There is generally good correlation between maternal blood lead levels during pregnancy and newborn levels (Schell, Denham et al. 2003).

C - Ethical Considerations

The protocol will be submitted for review to all appropriate human subjects research ethics committees. The informed consent forms are attached (Appendix 3). Withdrawal forms are also available (Appendix 13).

23. Risks

Blood samples will be collected during regularly scheduled clinic visits and by trained nurses. The following risks are known for venipuncture: hematoma, swelling and inflammation at the site, persistent bleeding, vasovagal response(dizziness, sweating, coldness of skin, numbness and tingling of hands and feet, nausea, vomiting, possible visual disturbance, syncope, and injury from fainting). Rare adverse effects: thrombosis and infection which results in thrombophlebitis. Special precautions will be taken: use of sterile equipment, use of a trained nurse to perform the blood draw with a physician on call in case an adverse affect occurs.

For the breast milk and urine collection, participation in this research is no greater than that encountered by the participant in her every day life. Thus, the human milk and urine collection is regarded as within the range of minimal risk to the participants as outlined in section C1 of the tri-council policy statement (TCPS-(Tri-Council-Policy-Statement) 2005). She may be inconvenienced by manual expression of milk. If the mother is unfamiliar with the technique, she could consult the pamphlet on how to manually express milk which is included in the kit, or alternatively, contact the nurse for additional assistance. The amount of milk required is not considered excessive since the mother is asked to supply the milk over a number of feedings and for several days. She is asked to express the milk during or after she has fed her child to ensure that the infant is not deprived in favour of participating in the study. If the child is hungrier on days planned for the collection of human milk for the study, the mother may delay collection by a day or two.

Hair collection will be performed by the trained nurse upon collection of the milk sample at the participant's home. A 5 mm bundle or approximately 50 hairs (equivalent to a diameter of a pencil) will be isolated and cut from the occipital region, ensuring minimum damage to the participants'

aesthetics. There are minimum risks associated with the sampling of the hair. Potential exists from mishandling of scissors resulting in laceration.

24. Benefits

The benefits of participating in this study include individual altruism and a benefit to others. Each participant will also benefit from receiving through her doctor her individual results of heavy metal analyses (lead, mercury and cadmium) if they are higher than health-based guidelines that are scientifically proven to be significant for the health of the participant and there are preventive measures or treatments. This information will be given along with some material develop at Health Canada on these chemicals. The benefit of this research will be reaped by the collective since the information may contribute to the improvement of public health, food safety and the environment. This study will also be meeting obligations to the larger international community (re: Stockholm convention).

25. Confidentiality

Confidentiality of the information will be met by assigning numerical codes that will be used on the questionnaires and on all biospecimen containers. The names and addresses of participants will be kept separate from the body of the questionnaire and from the sample labels, and will not be accessible to any individual other than the site investigators and their research staff. The questionnaires and the informed consents will be stored under lock and key and accessed only by the site investigators. The biological samples will be used for analysis of the substances that are outlined in this proposal. Residual samples, as proposed earlier, will be stored for future analyses of related substances and will be stored for 30 years. The samples will not be used for scientific investigations in any way associated with genetic profiling.

26. Voluntary participation

The program is entirely voluntary. The mothers have the right to withdraw from the program at any time without penalty or loss of benefits to which she is otherwise entitled.

27. Reporting results back to mothers

In communicating the individual results, we need to consider the risks and benefits as well as the variability and uncertainties in the underlying science. In spite of efforts at quantifying the various parameters, particularly for environmental chemicals, we are unable to interpret concentrations at the individual level. Further, the turn around time could be as long as three to four years due to the lengthy laboratory analyses. For those environmental chemicals for which there are current guidelines (e.g., lead, mercury), and a shorter turn around time, results will be shared with the participant through her doctor along with information on potential sources of exposure if they are higher than health-based guidelines that are scientifically proven to be significant for the health of the participant and there are preventive measures or treatments. Further, cognizant of the benefits conferred to the infant by breastfeeding; caution will be exercised regarding any recommendation that would discourage breastfeeding. The hospital ethics committees were of the opinion that releasing environmental chemical biomonitoring results to participants where the health significance was unknown would result in a removal of the researchers' accountability to do no harm and the mother would be left with results that could not be interpreted and which could cause her unnecessary anxiety. Furthermore, the hospital ethics committees were of the view that communicating these results posed a problem in terms of fairness, (in the sense of equitable use of health resources). These committees felt that the participants' preferences to obtain their

results and the potential benefits to them were outweighed by the potential burden on healthcare resources that benefit the whole society, as the sharing of results could lead to an increased number of women consulting their physicians for tests where health-based thresholds are not known. For these reasons, the ethics committees determined that the test results for those environmental chemicals whose toxicity threshold was unknown should not be communicated to participants, even if they so requested (Haines et al., 2010).

28. Publishing

Collective (not individual) results will be published in government reports and in peer reviewed journals. Names and addresses of the participants will not feature on any report or publication.
Results will be presented at scientific conferences and in scientific publications. Once peer reviewed, the results will also be shared within Health Canada and the federal/provincial/territorial health and environment committee developing risk assessment and management guidelines for heavy metals, as well as the Canadian and American Pediatric Societies so that these results can be translated into improved maternal and child health.

III - Timeline

Women will be recruited into the study over a 2 to 3 year recruitment period with each site expected to recruit between 100 and 150 women per year. Biological specimens will be collected, processed and stored in batches until analysis through early 2012. Statistical analysis and manuscript preparation will be done in the 5th year of support.

IV - Public Health Relevance

This study offers a very unique opportunity to examine the reproductive toxicity of heavy metals in a large prospective Canadian population of pregnant women. An added benefit is to study the role of nutritional and genetic factors in reducing the risk of heavy metal toxicity during pregnancy for the mother and her infant. Based on US data, blood lead levels in children have declined; however, new research suggests that even at levels below the current Health Canada and CDC intervention level of 10 µg/dl, health effects are observed. The added endogenous exposures during pregnancy from bone stores and increased susceptibility of the fetus to environmental toxicity are sources of concern. Further, postnatal follow-up to collect human milk samples for measurements of nutrients, environmental chemicals and immunoprotective constituents enables the creation of a unique and substantial data base of prenatal (blood) and postnatal (milk) exposure data which will serve to re-examine existing policy related to infant and maternal health. Overall, the results of this research are expected to strengthen health risk assessments (including promotion of breastfeeding) and to support measures to reduce release of contaminants into the environment, and limit exposure of the general population.

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VI - Appendices

Appendix 1: Proposal Budget

Appendix 2: Questionnaires

Appendix 3: Consent Forms

Appendix 4: Shipping instructions for biological Samples

Appendix 5: Lab procedures

Appendix 6: Recruitment Poster

Appendix 7: Recruitment Pamphlet

Appendix 8: Instruction Sheet for Collection of Human Milk

Appendix 9: Recruitment Log Book

Appendix 10: MIREC Results Reporting

Appendix 11: MADO Results Reporting

Appendix 12: FFQ Validation Protocol

Appendix 13: Withdrawal Forms

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 1A - Visit 1 - Baseline Questionnaire *(between 6 0/7 and 13 6/7 weeks)*

Center | ID | Monogram | Day | Month | Year

INTRODUCTION SECTION

ELIGIBILITY CHECK

Inclusion criteria

If you answer "YES" to all of the Inclusion Criteria, the woman could be ELIGIBLE for the study. Next, complete the exclusion criteria.

	Yes	No
1. The woman is pregnant between 6 0/7 and 13 6/7 completed weeks (If early ultrasound and LMP dates differ by ≤ 7 days, base GA estimate on LMP date; if > 7 days, use early ultrasound)	☐	☐
2. Age ≥ 18 years	☐	☐
3. Speaks French or English	☐	☐
4. Plans to deliver in a participating study hospital and donate cord blood to the study	☐	☐
5. The woman is able to understand and sign a consent form	☐	☐

Exclusion criteria

If you answer "NO" to all of the Exclusion Criteria, the woman is ELIGIBLE for the study. If there is at least one "YES", the woman is NOT ELIGIBLE for the study.

	Yes	No
6. Known major fetal abnormalities, including chromosomal anomalies	☐	☐
7. Has one of the following conditions:		
7.1 Renal disease with altered renal function (creatinine ≥ 2 times the upper limit of the normal range value)	☐	☐
7.2 Any collagen vascular disease (incl. lupus erythromatosus, scleroderma)	☐	☐
7.3 Active or chronic hepatitis	☐	☐
7.4 Epilepsy	☐	☐
7.5 Heart disease	☐	☐
7.6 Serious pulmonary disease	☐	☐
7.7 Cancer	☐	☐
7.8 Hematologic disorder	☐	☐
7.9 Threatened abortion (Woman with a previous bleeding in the 1st trimester can be included if there is a viable fetus at the time of recruitment, confirmed by ultrasound)	☐	☐
7.10 Illicit drug use	☐	☐

Eligibility

If you answered "YES" to all of the Inclusion Criteria and "NO" to all of the Exclusion Criteria, the woman is ELIGIBLE

CONSENT FORM

8. Date the eligible woman signed the main consent form dd mmm yyyy ☐☐ ☐☐☐ ☐☐☐☐

9. Did she agree to participate in the biobank for future research? ☐ Yes ☐ No

INTRODUCTION SECTION(CONTINUED)

I want to start by thanking you for your help with this study. I will be asking you questions about your education, income, places you lived, your diet during pregnancy, chemicals you might have been exposed to and drugs or medication you might have taken during your pregnancy. If a question makes you feel uncomfortable you don't have to answer it. We would appreciate you being as honest as possible in your answers. Do you have any questions before we begin?

SOCIODEMOGRAPHIC CHARACTERISTICS

10. What is your year of birth? *yyyy* ☐☐☐☐

11. First 3 characters of your postal code ☐☐☐

12. Highest education level achieved

☐ 0 Grade 8 or less ☐ 1 Some High School ☐ 2 High School Diploma
☐ 3 Some College Classes ☐ 4 College Diploma ☐ 5 Trade School Diploma
☐ 6 Undergraduate University Degree ☐ 7 Graduate University Degree (MSc, PhD)

13. Are you currently attending school? ☐ 1 Yes ☐ 0 No

14. People in Canada come from many racial or cultural groups. You may belong to more than one group on the following list. Are you... (check all that apply)

14.1 White ☐ 14.2 Chinese ☐ 14.3 South Asian (East Indian, Sri Lankan, Pakistani, etc.) ☐
14.4 Black ☐ 14.5 Filipino ☐ 14.6 Southeast Asian (Vietnamese, Cambodian, Malaysian, Laotian, etc.) ☐
14.7 Latin American ☐ 14.8 Arab ☐ 14.9 West Asian (Iranian, Afghan, etc.) ☐
14.10 Japanese ☐ 14.11 Korean ☐ 14.12 Aboriginal (North American Indian, Métis, or Inuit [Eskimo]) ☐
14.13 Other ☐ 14.13.1 If other, specify_____ 14.14 Refuse to answer ☐ 4.15 Don't know ☐

15. Marital Status

☐ 0 Married ☐ 1 Same partner for 1 year or more ☐ 2 Widowed
☐ 3 Divorced ☐ 4 Separated ☐ 5 Single
☐ 6 Other 15.1 If other, specify: _____

16. Living status

☐ 0 Live alone ☐ 1 With roommate ☐ 2 With spouse ☐ 3 With parents
☐ 4 Other 16.1 If other, specify: _____

17. From all sources in Jan-Dec of last year, what was your annual household income before taxes? *(including other sources of income, help from family or friends)*

☐ 0 Less than $10 000 ☐ 1 $10 001 – $20 000 ☐ 2 $20 001 – $30 000
☐ 3 $30 001 – $40 000 ☐ 4 $40 001 – $50 000 ☐ 5 $50 001 – $60 000
☐ 6 $60 001 – $70 000 ☐ 7 $70 001 – $80 000 ☐ 8 $80 001 – $100 000
☐ 9 More than $100 000 ☐ 98 Don't know ☐ 99 Refuse to answer

18. How many people were supported by that income? ☐☐

INTRODUCTION SECTION (CONTINUED)

SOCIODEMOGRAPHIC CHARACTERISTICS (CONTINUED)

19. In what country were you born?

☐ 1 Canada ☐ 2 United States ☐ 3 Mexico ☐ 4 China ☐ 98 Don't know
☐ 5 Other 19.1 If other, specify _____

Now I'm going to ask you some questions regarding your past pregnancies (record answers in table below).
A- If you were pregnant before the current pregnancy, for each one, what was the result of your pregnancy? (Probe: Did you have twins? Did you have a single baby? Was the baby born stillborn?)
B- On what date did your pregnancy end? (Probe: What was your (1st, 2nd, etc.) child's birthday? Date of miscarriage or abortion?) (month/year)
C- How many weeks pregnant were you when the pregnancy ended?
D- What was the baby's sex (if liveborn or stillborn)?
E- What was the baby's birth weight (if liveborn or stillborn)?
F- Is the child living?

OBSTETRICAL HISTORY

20. Have you ever been pregnant before this pregnancy? (If no, go to CURRENT PREGNANCY) ☐ 1 Yes ☐ 0 No

20.1 If yes, how many pregnancies, including the current pregnancy ☐☐
(Probe: No matter what happened with the pregnancy)

21. Description of each prior pregnancy and each baby (from the first to the most recent)

*Use the following codes for answering column A. birth outcome:

1. Live single birth
2. Live multiple birth
3. Stillbirth (≥ 20 weeks) *(Ask A, B, C, D, E)*
4. Spontaneous abortion (< 20 weeks) *(Ask A, B, C)*
5. Elective abortion *(Ask A, B, C)*
6. Ectopic pregnancy *(Ask A, B, C)*
7. Molar pregnancy *(Ask A, B, C)*

	A. Outcome	B. Date		C. Gest. Age	D. Gender	E. Weight				F. Still alive
	Code	*mmm*	*yyyy*	*Nber of* weeks	*Male=0 Female=1*	*Pounds*	*Oz*	***or***	*Grams*	*No=0 Yes=1*
21.1	☐	☐☐☐	☐☐☐☐	☐☐	☐	☐☐	☐☐	**or**	☐☐☐☐	☐
21.2	☐	☐☐☐	☐☐☐☐	☐☐	☐	☐☐	☐☐	**or**	☐☐☐☐	☐
21.3	☐	☐☐☐	☐☐☐☐	☐☐	☐	☐☐	☐☐	**or**	☐☐☐☐	☐
21.4	☐	☐☐☐	☐☐☐☐	☐☐	☐	☐☐	☐☐	**or**	☐☐☐☐	☐
21.5	☐	☐☐☐	☐☐☐☐	☐☐	☐	☐☐	☐☐	**or**	☐☐☐☐	☐
21.6	☐	☐☐☐	☐☐☐☐	☐☐	☐	☐☐	☐☐	**or**	☐☐☐☐	☐
21.7	☐	☐☐☐	☐☐☐☐	☐☐	☐	☐☐	☐☐	**or**	☐☐☐☐	☐
21.8	☐	☐☐☐	☐☐☐☐	☐☐	☐	☐☐	☐☐	**or**	☐☐☐☐	☐
21.9	☐	☐☐☐	☐☐☐☐	☐☐	☐	☐☐	☐☐	**or**	☐☐☐☐	☐
21.10	☐	☐☐☐	☐☐☐☐	☐☐	☐	☐☐	☐☐	**or**	☐☐☐☐	☐

22. In a previous pregnancy, have you suffered from gestational hypertension (Probe: preeclampsia)?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know

INTRODUCTION SECTION (CONTINUED)

CURRENT PREGNANCY

Now I'm going to ask you some questions regarding your current pregnancy.

23. When was the first day of your last menstrual period *dd mmm yyyy*
(the day when your period began)

24. Have you had an ultrasound (US) yet? ☐ Yes ☐ No If no, go to Question 25

Important: if the woman is planning to have her 1st ultrasound done in a non participating facility, ask her to bring a copy of the report at next visit.

If *yes* 24.1 Is the US report available at this visit? ☐ Yes ☐ No
If no, go to Question 25. You will be able to record this information in Questionnaire 2. If the US was done in a non participating facility, ask the woman to bring a copy of her report at visit 2.

If *yes* 24.1.1 Date of the US *dd mmm yyyy*

24.1.2 Gestational age (GA) written in the report ☐☐ Weeks ☐ Days/7

24.1.3 Dating with the crown-rump length (CRL)? ☐ Yes ☐ No

24.1.4 Number of fetuses ☐ Don't know ☐

25. Define the accurate GA on the day of visit 1: if early ultrasound and LMP dates differ by ≤ 7 days, base GA on LMP date; if they differ by > 7 days, use early ultrasound result
☐☐ Weeks ☐ Days/7

25.1 GA at visit 1 estimated by: ☐ Last Menstrual Period **or** ☐ Early Ultrasound Examination

25.2 Expected date of confinement *dd mmm yyyy*

26. Have you and the baby's father ever tried to become pregnant over a period of at least a year without success?
☐ Yes ☐ No

27. How long did it take you to get pregnant with this pregnancy? ☐☐ Months

28. Did you use any assisted reproductive technologies or ovulation inducing drugs to become pregnant?
☐ Yes ☐ No ☐ Refused to answer

If yes 28.1 Do you remember which one(s)? ☐ Yes ☐ No

If *yes*, specify:

28.1.1 Artificial insemination ☐ Yes ☐ No If *yes*: 28.1.1.1 ☐ Your spouse's sperm **or** ☐ Donor's sperm

28.1.2 In Vitro Fertilization ☐ Yes ☐ No If *yes*: 28.1.2.1 ☐ Your spouse's sperm **or** ☐ Donor's sperm

28.1.3 Ovulation inducing agents ☐ Yes ☐ No

28.1.4 Oocyte donation ☐ Yes ☐ No

28.1.5 Other ☐ Yes ☐ No If *yes*: 28.1.5.1 Specify _____

INTRODUCTION SECTION (CONTINUED)

CURRENT PREGNANCY (CONTINUED)

29. Thus far, have you had any complications with this pregnancy? ☐ Yes ☐ No

If *yes:* 29.1 What complications have you had with this pregnancy?

☐ Repeated vomiting with weight loss ☐ Vaginal Bleeding

☐ Other 29.1.1 If other, specify _____

30. Before this pregnancy, what was the last type of birth control that you and the baby's father used? (check all that apply)

30.1 None	☐	If None, go to question 32.
30.2 IUD	☐	
30.3 Male condom	☐	
30.4 Female condom	☐	
30.5 Contraceptive pill	☐	
30.6 Spermicidal foam	☐	
30.7 Periodical abstinence (Rhythm)	☐	
30.8 Other	☐	30. 8.1 If other, specify _____

31. Did you stop this method of birth control before the pregnancy started, or was there a birth control failure?

☐ Stopped before ☐ Birth control failure ☐ Not clear

FAMILY HISTORY

32. Did your biological mother have high blood pressure during any of her pregnancies?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know

32.1 If *yes*, did your biological mother have high blood pressure when she was pregnant with you?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know

33. Do you have at least one biological sister who had a pregnancy which lasted at least 20 weeks?

☐ 1 Yes ☐ 0 No ☐ 97 N/A ☐ 98 Don't know

33.1 If *yes*, did any of your biological sisters have high blood pressure during any of her/their pregnancies?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know

INTRODUCTION SECTION (CONTINUED)

NON OBSTETRICAL HISTORY Do you have any chronic medical condition(s)? (Any condition that treatment can alleviate, but not cure)

A. Condition	B. Year of Diagnosis	C. Current Treatment/Medication (When possible use generic name, except for combination products)
34. High Bloood Presure ☐1 Yes ☐0 No	☐☐☐☐	
35. Diabetes ☐1 Yes ☐0 No	☐☐☐☐	35.1 C Diet ☐1Yes ☐0No 35.2 C Hypoglycemic agents ☐1Yes ☐0No 35.3C Insulin ☐1Yes ☐0No

36. Other chronic condition(s) ☐1 Yes ☐0 No
If yes, list all conditions and specify treatment(s) in C column (more than 1 treatment possible per line):

36.1	☐☐☐☐	36.1C
36.2	☐☐☐☐	36.2C
36.3	☐☐☐☐	36.3C
36.4	☐☐☐☐	36.4C
36.5	☐☐☐☐	36.5C

SPECIFIC MEDICATION

37. Do you take ASA regularly ☐1 Yes ☐0 No

37.1 If *yes*, explain the indication _____

NUTRITIONAL SUPPLEMENTS Over the last 3 months, did you take vitamin/mineral supplements?

A. SUPPLEMENTS	B. Name of the products (when possible use generic name, except for combination products)
38. Prenatal multivitamin preparation ☐1 Yes ☐0 No	38.1B 38.2B 38.3B
39. Folic Acid Supplements ☐1 Yes ☐0 No	39.1B 39.2B 39.3B
40. Other supplements (e.g., iron, calcium, B-Carotene, vitamin C, vitamin E, fish oil) ☐1 Yes ☐0 No	40.1B 40.2B 40.3B

INTRODUCTION SECTION (CONTINUED)

EMPLOYMENT STATUS

41. Since the beginning of your pregnancy, have you been employed *(Probe: had any type of job)* ☐ 1 Yes ☐ 0 No
If no, go to ENVIRONMENTAL EXPOSURES SECTION - PART A

If *yes*, for any jobs you have held during this pregnancy, please tell me the type of business and what type of work you do.

41.1 Job 1 ☐ 0 Part time ☐ 1 Full time

41.1.1 Type of business_____ 41.1.2 Type of work _____

41.2 Job 2 ☐ 0 Part time ☐ 1 Full time

41.2.1 Type of business_____ 41.2.2 Type of work _____

41.3 Job 3 ☐ 0 Part time ☐ 1 Full time

41.3.1 Type of business_____ 41.3.2 Type of work _____

42. During your pregnancy, have there been any non-routine work events, like chemical leaks or spills in your workplace?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know

42.1 If *yes*, what chemical spilled? _____

42.2 Were you directly exposed to the chemical spill? ☐ 1 Yes ☐ 0 No ☐ 97 N/A ☐ 98 Don't know

43. Prior to or during your pregnancy, were you required or advised by any means to wear protective equipment such as clothing or a respirator (please specify the type of equipment and what it is supposed to protect you from)?

43.1 Prior to pregnancy ☐ 1 Yes ☐ 0 No ☐ 97 N/A ☐ 98 Don't know

43.1.1 If *yes*, specify type_____ 43.1.2 Specify what _____

43.1.3 How long ago? ☐ 0 <3 months ☐ 1 3-12 months ☐ 2 >12 months ☐ 98 Don't know

43. 2 During pregnancy ☐ 1 Yes ☐ 0 No ☐ 97 N/A ☐ 98 Don't know

43.2.1 If *yes*, specify type_____ 43.2.2 Specify what _____

44. During your pregnancy, how often do you wear protective equipment on the job? Would you say:

☐ 0 Always ☐ 1 2-3 times / week ☐ 2 1/week ☐ 3 1/month

☐ 4 Never ☐ 97 N/A ☐ 98 Don't know

☐ ☐ ☐ ☐

ENVIRONMENTAL EXPOSURES SECTION - PART A

☐ ☐ ☐

☐ ☐ ☐ ☐ ☐

Now I am going to ask you about other environmental exposures during your pregnancy. Please think about the ***current pregnancy,*** *either at work or at home.*

A. Have you been exposed to [INSERT EXPOSURE FROM TABLE BELOW] ? [RECORD ANSWER IN TABLE BELOW]

If Yes, ASK: ***If NO, ASK:***

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 1A – Visit 1 – Baseline Questionnaire *(between 6 0/7 and 13 6/7 weeks)*

Center ☐☐ ID Monogram

B. Was your exposure direct? (Probe: Did you handle [INSERT EXPOSURE]?)

C. How often are you exposed to [INSERT EXPOSURE] ? Would you say. . .

D. Did the exposure to [INSERT EXPOSURE] occur while you were at work?

E. Within the past 5 years. Have you been exposed to [INSERT EXPOSURE]?

F. How long ago were you exposed?

EXPOSURE	Since the beginning of this pregnancy				Before this pregnancy	
	A. Exposure During Pregnancy	B. Direct Exposure Doing it yourself or direct contact with the substance	C. Frequency	D.Exposure at work	E. Ever exposed	F. How long ago were you exposed
1.Coal products from hot asphalt or tar roofing material	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
2.Carbon black from copying or printing machines	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
3. Clothing Dyes	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
4. Fresh oil-based paint	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
5. Fresh latex or acrylic paint	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know

ENVIRONMENTAL EXPOSURES SECTION - PART A (CONTINUED)

EXPOSURE	Since the beginning of this pregnancy				Before this pregnancy	
	A. Exposure During Pregnancy	B. Direct Exposure Doing it yourself or direct contact with the substance	C. Frequency	D.Exposure at work	E. Ever exposed	F. How long ago were you exposed
7. Solvents	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
Cosmetic Products						
8. Hair dyes	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
9. Hair relaxers	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
10. Perm solutions	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
11. Hair sprays or gels	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
12. Nail care products (for application or removal)	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
13. Makeup (for example lip liner, lipstick, foundation, mascara, blush, eyeliner, eye shadow)	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know

ENVIRONMENTAL EXPOSURES SECTION - PART A (CONTINUED)

EXPOSURE	Since the beginning of this pregnancy				Before this pregnancy	
	A. Exposure During Pregnancy	B. Direct Exposure Doing it yourself or direct contact with the substance	C. Frequency	D.Exposure at work	E. Ever exposed	F. How long ago were you exposed
14. Fragrances, perfumes or colognes	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 1/ week ☐ <1 month ☐ 2-3/week ☐ 1/ month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
15. Skin lotions or moisturizers	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 1/ week ☐ <1 month ☐ 2-3/week ☐ 1/ month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
Pesticides for:						
16. Lawn/garden weeds	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 1/ week ☐ <1 month ☐ 2-3/week ☐ 1/ month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
17. Lawn/garden insects	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 1/ week ☐ <1 month ☐ 2-3/week ☐ 1/ month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
18. House plant insects	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 1/ week ☐ <1 month ☐ 2-3/week ☐ 1/ month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ < 3 months ☐ 3- 12 months ☐ >12 months ☐ Don't know
19. Pet fleas	☐ Yes ☐ No	☐ Yes ☐ No	☐ Daily ☐ 2-3/week	☐ Yes ☐ No	☐ Yes ☐ No	☐ < 3 months

ENVIRONMENTAL EXPOSURES SECTION - PART B

1. Have any renovations been done in your home during this pregnancy?

☐ 1 Yes ☐ 0 No

If *yes*, check all that apply:

1.1 ☐ Carpets – installed new/replaced old
1.2 ☐ Commercial cleaning – drapes, carpets or furniture
1.3 ☐ Furniture – restoring/refinishing
1.4 ☐ Insulation – replaced, installed new or removed old
1.5 ☐ Landscaping
1.6 ☐ Paint – removed by scraping, sanding or heat gun
1.7 ☐ Paint – removed using chemical strippers
1.8 ☐ Painting – Exterior – with spray paint (in aerosol container)
1.9 ☐ Painting – Interior – with spray paint (in aerosol container)
1.10 ☐ Painting – Interior/Exterior – with oil-based (alkyd) paints
1.11 ☐ Painting – Interior/Exterior – with latex or acrylic paints
1.12 ☐ Vinyl Flooring – replaced
1.13 ☐ Wallpaper – removed/added
1.14 ☐ Windows – applied caulking, grout or sealant
1.15 ☐ Wood Flooring – refinishing
1.16 ☐ Other 1.16.1 If other, specify_____

2. Within the past 12 months, have you had any mercury-silver (also known as amalgam) dental fillings replaced?

☐ 1 Yes ☐ 0 No

3. Currently, how many mercury-silver dental fillings do you have?

☐ 0 0 ☐ 1 1-4 ☐ 2 5-9 ☐ 3 10-14 ☐ 4 15+ ☐ 98 Don't know

ENVIRONMENTAL EXPOSURES SECTION - PART B (CONTINUED)

4. The following question refers to activities or hobbies. Have you or anyone living in your home done any of the following **in the past 3 months**?

Activity/Hobby	A. Participant	B. Anyone living in home
4.1 Painting	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.2 Pottery or ceramics	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.3 Candle making	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.4 Stained glass soldering	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.5 Welding or soldering	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.6 Print making	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.7 Auto body repair	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.8 Developing of photographs (darkroom lab)	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.9 Furniture stripping/refinishing	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.10 Working with dyes, fibers and fabrics	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.11 Jewellery, hollowware and enamelling	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.12 Metal working	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.13 Electronics	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.14 Automotive mechanical work	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.15 Silk screening	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.16 Wood working	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.17 Crafts involving glues, solvents	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.18 Use of chemicals to control weeds in lawn or garden	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.19 Use of chemicals to control insects or disease in lawn, garden or interior house plants	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.20 Use of chemicals to control pests indoors (e.g., rodents, fleas)	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.21 Other 4.21.1 If other, specify:	☐ 1 Yes ☐ 0 No _____	☐ 1 Yes ☐ 0 No _____

ENVIRONMENTAL EXPOSURES SECTION - PART B (CONTINUED)

5. Do you have or have you previously had a carpet or upholstery in your home or in your car that was treated with a non-stick or stain-resistant material? ☐ 1 Yes ☐ 0 No ☐ 98 Don't know

6. In the last month (30 days), have you used cooking vessels designed for stove and/or oven use that are made of any of the following material types?

6.1 Non stick	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.2 Cast iron	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.3 Glazed ceramic	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.4 Stainless steel	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.5 Aluminium	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.6 Other metallics (such as bronze or copper)	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.7 Other	☐ 1 Yes	☐ 0 No	☐ 98 Don't know

6.7.1 If other, specify _____

7. In the last month (30 days), have you used any of the following vessels for heating food or drinks in the microwave?

7.1 Non stick	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
7.2 Glazed ceramic	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
7.3 Plastic	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
7.4 Disposable food containers (e.g., margarine tubs)	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
7.5 Other	☐ 1 Yes	☐ 0 No	☐ 98 Don't know

7.5.1 If other, specify _____

SMOKING SECTION - PART A

SMOKING HISTORY / ACTIVE SMOKER *Now I'd like to ask you about your smoking history. Please include cigarettes that are ready-made as well as cigarettes that you make yourself.*

1. Have you ever smoked at least 100 cigarettes over your lifetime (about 4 packs)?

☐ 1 Yes ☐ 0 No (if no, go to SMOKING SECTION - PART B)

2. At the present time, do you smoke cigarettes daily, occasionally or not at all?

☐ 0 Daily (GO TO QUESTION 5) ☐ 1 Occasionally (GO TO QUESTION 3) ☐ 2 Not at all (GO TO QUESTION 7)

3. On the days that you do smoke, how many cigarettes do you usually smoke?

☐☐ 0 Cigarettes ☐ 98 Don't know ☐ 99 Refused to answer

4. In the past month, on how many days have you smoked one or more cigarettes?

☐☐ 0 Days (GO TO QUESTION 6) ☐ 98 Don't know ☐ 99 Refused to answer

5. How many cigarettes do you smoke each day now?

☐☐ 0 Cigarettes ☐ 98 Don't know ☐ 99 Refused to answer

6.A What brand do you usually smoke? (*check only one – if more than one, check "No regular brand"*)

☐ 0 Belvedere Select (Extra Mild)
☐ 1 Number 7
☐ 2 Canadian Classics
☐ 3 Number 7 Blue (Light)
☐ 4 Canadian Classics Silver (Light)
☐ 5 Number 7 Silver (Extra Mild)
☐ 6 Canadian Classics White (Extra Light)
☐ 7 Peter Jackson Full Flavour
☐ 8 DuMaurier
☐ 9 Peter Jackson Select Flavour (Light)
☐ 10 DuMaurier Distinct (Light)
☐ 11 Peter Jackson Mellow Flavour (Extra Light)
☐ 12 DuMaurier Premiere (Extra Light)
☐ 13 Player's Original Flavour
☐ 14 DuMaurier Prestige (Ultra Light)
☐ 15 Player's Rich Flavour (Light)
☐ 16 Export "A" Medium
☐ 17 Player's Smooth Flavour (Extra Light)
☐ 18 Export "A" Smooth (Light)
☐ 19 Cigarettes from First Nations/Native Reserve
☐ 20 Export "A" Ultra Smooth (Ultra Light)
☐ 21 No regular brand (*check this if the patient smokes multiple brands)*
☐ 22 Matinée Slims (Extra Mild)
☐ 23 Other 6.A.1 If other, specify _____

6.B Specify the size:

☐ 0 King Size
☐ 1 Regular Size
☐ 2 100's
☐ 3 Other 6.B.1 If other, specify _____

SMOKING SECTION - PART A *(continued)*

SMOKING HISTORY / ACTIVE SMOKER *(continued)*

7. At what age did you smoke your first whole cigarette? ☐☐ Years

8. Have you stopped smoking? ☐ Yes ☐ No

8.1 If *yes*, when did you stop smoking?

☐ When I knew I was pregnant → 8.1.1 Date of cessation *dd mmm yyyy* ☐☐☐☐☐ ☐☐☐☐

☐ < 1 year ago ☐ ≥ 1 and < 2 years ago (go to SMOKING SECTION - PART B)

☐ ≥ 2 and < 3 years ago (go to SMOKING SECTION - PART B) ☐ ≥ 3 years ago (go to SMOKING SECTION - PART B)

☐ Don't know ☐ Refuse to answer

9. During the past 3 months, have you stopped smoking for at least 24 hours because you were trying to quit?

☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer

9.1 If *yes*, how many times ☐☐ ☐ Don't know ☐ Refuse to answer

10. During the past 3 months, did you try any of the following to quit smoking?

10. 1 Nicotine patch ☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer

10. 2 Nicorettes or other nicotine gum, lozenge or candy ☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer

10. 3 Medication such as Zyban ☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer

11. Does your doctor know that you [smoke/smoked] cigarettes?

☐ Yes ☐ No (go to SMOKING SECTION - PART B)

☐ Don't know (go to SMOKING SECTION - PART B) ☐ Refuse to answer (go to SMOKING SECTION - PART B)

12. During the past 3 months, did your doctor advise you to quit smoking?

☐ Yes ☐ No (go to SMOKING SECTION - PART B) ☐ Don't know ☐ Refuse to answer

13. During the past 3 months, did your doctor give you any specific help or information to quit smoking?

☐ Yes ☐ No (go to SMOKING SECTION - PART B) ☐ Don't know ☐ Refuse to answer

13.1 If *yes*, what type of help did the doctor give?

☐ Referral to a one-on-one cessation program ☐ Referral to a group cessation program

☐ Recommended use of nicotine patch or nicotine gum ☐ Recommended Zyban or other medication

☐ Provided self-help information (e.g., pamphlet, referral to Website) ☐ Own doctor offered counseling

☐ Other 13.1.1 If other, specify _____

SMOKING SECTION - PART B

EXPOSURE TO SECOND-HAND SMOKE

1. Including both household members and regular visitors, does anyone smoke inside your home, every day or almost every day? Note : Include cigarettes, cigars and pipes.

☐ 1 Yes ☐ 0 No (Go to question 4) ☐ 98 Don't know ☐ 99 Refuse to answer

2. How many people smoke inside your home every day or almost every day? Note: Include household members and regular visitors

☐☐ 0 People

3. On a typical day, how many cigarettes are smoked inside your home?

☐ 0 1 to 10 ☐ 1 11 to 20 ☐ 2 21 to 30 ☐ 3 31 to 40 ☐ 4 41 or more
☐ 98 Don't know ☐ 99 Refuse to answer

4. In the past month, were you exposed to second-hand smoke, every day or almost every day, in a car or other private vehicle?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

5. During your pregnancy, has anyone in your workplace smoked in your presence? (including breaks, lunch)

☐ 1 Yes ☐ 0 No ☐ 97 N/A ☐ 98 Don't know ☐ 99 Refuse to answer

6. During your pregnancy, were you exposed to second-hand smoke in public places?
(such as bars, arenas, restaurants or bingo halls)

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

DRINKS AND ALCOHOL CONSUMPTION SECTION *Now some questions about drinks and alcohol.*

1. Since the beginning of your pregnancy, how much did you drink of the following? (unless otherwise specified, 1 glass=8 oz, 1 cup=6 oz)

1.1 Water (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.2 Milk (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.3 Regular coffee (cups)	☐ None	☐☐	cups /	☐ Day ☐ Week ☐ Month
1.4 Decaffeinated coffee (cups)	☐ None	☐☐	cups /	☐ Day ☐ Week ☐ Month
1.5 Regular tea (cups)	☐ None	☐☐	cups /	☐ Day ☐ Week ☐ Month
1.6 Green tea (cups)	☐ None	☐☐	cups /	☐ Day ☐ Week ☐ Month
1.7 Herbal tea (cups)	☐ None	☐☐	cups /	☐ Day ☐ Week ☐ Month
1.8 Apple juice (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.9 Grape juice/cocktail (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.10 Other fruit juice (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.11 Vegetable juice (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.12 Other caffeinated beverages (e.g., colas, iced tea/coffee, chocolate, some sport/energy drinks) (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month

2. During the past 3 months, have you had a drink of beer, wine, liquor or any other alcoholic beverage?

☐ Yes ☐ No (if no, go to RESIDENCE SECTION) ☐ Refuse to answer

If *yes*, how much did you drink of the following? 2.1 White wine (1 glass = 4 oz)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month ☐ 3 Months
2.2 Red wine (1 glass = 4 oz)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month ☐ 3 Months
2.3 Beer (1 glass = 8 oz)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month ☐ 3 Months
2.4 Liquor (1 drink = 1 oz)	☐ None	☐☐	drinks /	☐ Day ☐ Week ☐ Month ☐ 3 Months

3. Thinking back over the past 3 months, did you ever consume 5 or more alcoholic drinks on one occasion?	☐ Yes	☐ No		
3.1 If *yes*, how many times		☐☐	times /	☐ Week ☐ Month ☐ 3 Months

RESIDENCE SECTION - PAST ADDRESSES LIST

Now I'm going to ask you some questions about each place you have lived in over the past 5 years. Starting with your current residence, "Please tell me. . . [RECORD ANSWERS IN TABLE BELOW]

A-C) your (previous) address?"
D) is this a house or apt?"
E-F) the dates during which you lived at this residence."
G) if you would consider this area predominantly urban, suburban, or rural."

Repeat Questions A- G until history is completed. Begin with the remarks: "Please tell me... "

Please provide your addresses over the **past 5 years**, starting with your current residence.

Residence	**A. Province or State**	**B. 1st 3 characters of postal code** (*If the woman doesn't remember, ask for the city's name and complete the white space)*	**C. Country**	**D. Type** **House** = 0 **Apt** =1	**E. From** (Date): mmm/yyyy	**F. To** (Date): mmm/yyyy	**G. Area** Urban= 0 Suburb =1 Rural= 2 Don't know= 3
1. Current							
2. Previous							
3. Previous							
4. Previous							
5. Previous							
6. Previous							
7. Previous							
8. Previous							
9. Previous							
10. Previous							

RESIDENCE SECTION - DETAILS

1. Would you describe the location where you spent most of your childhood as?

☐ 0 Densely populated urban city ☐ 1 Suburban city ☐ 2 Town or village
☐ 3 Rural ☐ 4 Farm ☐ 98 Don't know ☐ 99 Refuse to answer

2. Which of the following best describes your current home? Is it a …

☐ 0 Detached single family house ☐ 1 Duplex or townhouse ☐ 2 100% residential apartment building
☐ 3 Combined residential and commercial building ☐ 98 Don't know ☐ 99 Refuse to answer

3. Does your home have an attached garage?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

3.1 If yes, how many vehicles are usually parked in your garage? ☐☐

4. Is the truck or bus traffic on your street…

☐ 0 Light (occasional trucks/buses passing by) ☐ 1 Medium (many trucks/buses passing by)
☐ 2 Heavy (a continuous flow of trucks/buses) ☐ 98 Don't know

5. Do you ever notice paint chips or dust from paint in your home?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

6. Has your home/apartment had any repairs done in the last 2 years?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

If yes, what type of repairs/renovations occurred in your home? (check all that apply)

6.1 Leaky pipes ☐ 6.2 Holes/Cracks in the Ceiling/Wall ☐ 6. 3 Roof repairs ☐
6.4 Leaking basement ☐ 6. 5 Don't know ☐ 6.6 Other ☐ 6. 6.1 If other, specify_____

7. Now I'd like to ask some questions about the heating in your home. What is the main type of heating fuel in your home?

☐ 0 Natural Gas ☐ 1 Electric ☐ 2 Fuel oil ☐ 3 Coal ☐ 4 Wood
☐ 5 Other 7.1 If other, specify_____ ☐ 98 Don't know

8. How is your home heated (check all that apply)?

8.1 Radiator (steam or hot water) ☐ 8. 2 Forced hot air vents (furnace) ☐ 8. 3 Baseboard electric heaters ☐
8.4 Wood stove or fireplace ☐ 8. 5 Don't know ☐ 8. 6 Other ☐ 8. 6.1 If other, specify_____

RESIDENCE SECTION - DETAILS (CONTINUED)

9. Do you have a fireplace/woodstove? ☐ 1 Yes ☐ 0 No

9.1 If yes, what do you mostly burn in your fireplace/woodstove?

☐ 0 Natural gas ☐ 1 Propane ☐ 2 Wood or wood pellets ☐ 3 Coal ☐ 4 Corn pellets
☐ 5 Other 9.1.1 If other, specify _____
☐ 98 Don't know ☐ 99 Refuse to answer

10. Where do you get water for general household use (e.g. for showering and cleaning)? (check all that apply)

10.1 Public water system ☐ 10.2 Private well ☐ 10.3 I don't know ☐
10.4 Other source ☐ 10.4.1 If other, specify _____

11. When was your home or apartment built?

☐ 0 Prior to 1960 ☐ 1 1960-1970 ☐ 2 1971-1980 ☐ 3 1981-1990
☐ 4 1991-2000 ☐ 5 2001-2005 ☐ 6 2006 or more recently ☐ 98 Don't know

12. How long have you lived in this home?

☐ 0 < 1 year ☐ 1 1 – 5 years ☐ 2 6 – 10 years ☐ 3 > 10 years ☐ 98 Don't know

13. About the cooking appliances, which do you use indoors or outdoors at home (check all that apply)?

13.1 Electric stove ☐ 13.2 Gas stove ☐ 13.3 Wood stove ☐ 13. 4 Charcoal BBQ ☐
13.5 Propane/gas BBQ ☐

14. Do you burn candles in your home? ☐ 1 Yes ☐ 0 No

14.1 If yes, in the past month (30 days), how many times have you burned candles?

☐☐ times / month ☐ 98 Don't know

15. Do any rooms in your home have wall-to-wall carpets? ☐ 1 Yes ☐ 0 No

If yes, specify (check all that apply):

15.1 Your bedroom ☐ 15.2 Other bedrooms ☐ 15.3 Living room ☐
15.4 Family room ☐ 15.5 Dining room ☐ 15.6 Den/office ☐
15.7 Basement rec room ☐ 15.8 Other ☐ 15.8.1 If other, specify _____

16. Do you have a furnace ☐ 1 Yes ☐ 0 No ☐ 98 Don't know

16.1 If *yes*, how frequently are the filters changed in your furnace?

☐☐ times / ☐ 0 Month ☐ 1 Year ☐ 98 Don't know

ACTIVITIES SECTION

1. In a typical day, how much time (in minutes) do you spend on average? (Fill out all).

1.1 Walking	___ min.	1.2 On a motorcycle / scooter / moped	___ min.
1.3 Biking	___ min.	1.4 In a bus / tram	___ min.
1.5 In a car / taxi	___ min.	1.6 In a train / subway	___ min.

2. In a typical day, how much time (in minutes) do you spend on average directly exposed (within 20 ft) to any vehicle emitting exhaust vapours (e.g. at a bus stop)? ___ min.

3. During this pregnancy, please tell me your 3 most common means of transportation. Start by telling me what mode of transport you take the most, then the 2nd most, and finally, your 3rd most.

CODES
1. Walk
2. Bike
3. Taxi
4. Gas car, truck or SUV
5. Diesel car, truck or SUV
6. Bus /tram
7. Motorcycle / scooter / moped
8. Train/ subway
9. Other

Insert the code

3.1 Most Common ___ 3.1.1 If other, specify _____
3.2 2nd Most ___ 3.2.1 If other, specify _____
3.3 3rd Most ___ 3.3.1 If other, specify _____

4. Do you have a pet? ___ 1 Yes ___ 0 No If yes, specify.

[Write the number of dogs, cats and birds in the appropriate boxes; if the woman has other kinds, enter the total number in the box and specify in the text field **(e.g.:** Other 0 4 4.4.1 If other, specify 1 snake / 2 rabbits / 1 mouse)]

4.1 Dogs ___ 4.2 Cats ___ 4.3 Birds ___
4.4 Other ___ 4.4.1 If other, specify type and # for each _____

5. How frequently are the floors in your home washed by yourself using household cleaners?
___ Never ___ times / ___ 0 Week ___ 1 Month ___ 2 Year

6. In a typical week during this pregnancy, how many hours per week do you spend doing household cleaning?
___ 0 Less than 1 hour ___ 1 1 –2 hrs ___ 2 3 – 4 hrs ___ 3 5 –7 hrs ___ 4 more than 7 hrs

7. Which rooms in your home are the main ones where cleaning is carried out on a weekly basis (check all that apply)?

7.1 None ___	7.2 Your bedroom ___	7.3 Other bedrooms ___	7.4 Living room ___
7.5 Basement ___	7.6 Family room ___	7.7 Dining room ___	7.8 Kitchen ___
7.9 Bathroom(s) ___	7.10 Other ___	7.10.1 If other, specify _____	

DIET SECTION

During the last 2 weeks, how many times have you eaten [INSERT MEAT] that was cooked [INSERT COOKING METHOD] ?

	A. Fried	B. Broiled	C. BBQ/Charcoal broiled	D. Cooked so that it is blackened on the outside (by any cooking method)
1. Poultry	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week
2. Hamburger	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week
3. Steak	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week
4. Pork (not including sausage/bacon)	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week
5. Sausage or bacon	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week
6. Fish	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week

DIET SECTION (CONTINUED)

7. In the past 3 months, how often did you eat the following fish? (Note: each line must be completed)

7.1 Fish sticks	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.2 Tuna packaged in a can or pouch	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.3 Tuna steaks and/or fillets	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.4 Salmon packaged in a can or pouch	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.5 Fresh, frozen, and/or smoked salmon	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.6 Halibut	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.7 Rainbow trout	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.8 Lake trout	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.9 Cod	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.10 Whitefish	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.11 Tilapia	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.12 Shark	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.13 Marlin	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.14 Swordfish	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.15 Orange roughy	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.16 Escolar	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.17 Char	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month

DIET SECTION (CONTINUED)

7.18 Herring	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.19 Mackerel	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.20 Sardines	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.21 Flounder	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.22 Plaice	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.23 Sole	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.24 Pollock	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.25 Haddock	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.26 Other fish 7.26.1 If yes, specify_____	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.27 Other fish 7.27.1 If yes, specify_____	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month

8. Since the beginning of your pregnancy, how many servings of fruit or vegetables from your own garden have you consumed?

☐ None ☐☐ servings / ☐ Day ☐ Week ☐ Month

9. Since the beginning of your pregnancy, how frequently have you eaten coffee-flavoured yogurt or ice cream?

☐ Never ☐☐ times / ☐ Day ☐ Week ☐ Month

SUNLIGHT EXPOSURE SECTION

1. Would you say the natural colour of the inside of your upper arm is:
   ☐ Light (white, fair) ☐ Medium (olive, light brown, medium brown) ☐ Dark (dark brown, black)
2. During the past 4 weeks, how many days did you spend outside for at least 30 minutes between 9 am and 4 pm?
   ☐☐ Days ☐ None If None, go to question 3.
   2.1 When you were outside, how would you describe the coverage of your legs and arms with clothing?
   ☐ None ☐ Partial ☐ Total
   2.2 What was the Sun Protector Factor (SPF) of the main sunscreen that you used?
   (the one that you used on the largest exposed area of your body)
   ☐ 4 ☐ 8 ☐ 15 ☐ 30 ☐ 45
   ☐ Other 2.2.1 If other, specify_____ ☐ N/A (did not use any sunscreen)
3. During the past 4 weeks, did you use a sunlamp or tanning bed?
   ☐ Yes ☐ No

ANTHROPOMETRIC MEASUREMENTS SECTION

1. Pre-pregnancy weight (just before you became pregnant) ☐☐☐•☐ ☐ Kg ☐ Pounds ☐ Don't know
2. Weight measured at this visit ☐☐☐•☐ ☐ Kg ☐ Pounds
   If not, complete the PROTOCOL DEVIATION SECTION
3. Height measured at this visit ☐☐☐ cm
   If not, complete the PROTOCOL DEVIATION SECTION

BLOOD PRESSURE AND URINE COLLECTION SECTION

BLOOD PRESSURE MEASUREMENT DURING THIS VISIT (SITTING, LEFT ARM)

1. Have blood pressure measurements been taken? ☐ 1 Yes ☐ 0 No
   If no, complete the PROTOCOL DEVIATION SECTION

If yes, record 1.1 1st Measure ☐☐☐ / ☐☐☐ mm/Hg
systolic *diastolic*

One minute apart:

1.2 2nd Measure ☐☐☐ / ☐☐☐ mm/Hg
systolic *diastolic*

BLOOD PRESSURE AND URINE COLLECTION SECTION (CONTINUED)

URINE SAMPLE COLLECTION

Collect a minimum of 80 ml of urine into a 125ml Nalgene container

2. Collection of sample done (CC1) ☐ 1 Yes ☐ 0 No If no or less than expected, complete the PROTOCOL DEVIATION SECTION; if *yes:*

2.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 2.2 Time (24 hr clock) ☐☐ hr ☐☐ min

2.3 Number of hours since last urination (prior to this one) ☐☐ hr ☐☐ min

2.4 Processing and freezing of urine sample done ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If *yes* 2.4.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 2.4.2 Time (24 hr clock) ☐☐ hr ☐☐ min

URINE PROTEIN DIPSTICK TEST Transfer at least 5ml of urine from the 125ml Nalgene container into a 60ml urine container

3. Urine protein test by dipstick done ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

3.1 If *yes*, indicate the result obtained

☐ Zero ☐ 1+ or 30 mg/dL or 0.3 g/L ☐ 3+ or 300 mg/dL or 3.0 g/L

☐ Trace ☐ 2+ or 100 mg/dL or 1.0 g/L ☐ 4+ or 2000 mg/dL or 20 g/L

If protein on dipstick ≥ 1+, check in the patient chart if a 24-hour urine protein test has been ordered in the last seven days:

3.1.1 Was the 24-hour urine test ordered because of a positive dipstick? If not, inform to the patient's doctor the dipstick results and indicate that documentation of a 24-hour urine protein test is highly desirable for the study protocol. If he orders it, mark Yes. If not, specify the reason in the PROTOCOL DEVIATION SECTION.

☐ 1 Yes ☐ 0 No

If *yes*, probe: If the 24-hour urine protein test was ordered today, explain to the patient it should be done within the next 7 days and wait for the results to complete the rest of the section.

3.1.1.1 Indicate the result obtained ☐☐☐☐☐ mg/24 h **or** ☐☐ • ☐ g/L

3.1.1.2 Date test performed *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

BLOOD SAMPLE COLLECTION SECTION

1. Was the woman fasting? ☐ 1 Yes ☐ 0 No

2. Specimen collection done? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

2.1 If *yes* Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐ 2.2 Time (24 hr clock) ☐☐ hr ☐☐ min

2.3 Check tubes collected (3 x10 ml and 2 X 6 ml expected) If less specimens than expected, complete the PROTOCOL DEVIATION SECTION

☐ 10 mL K2-EDTA (CC2) ☐ 10 mL K2-EDTA (CC3) ☐ 6 mL K2-EDTA (CC4) ☐ 6 mL blue tube (CC5) ☐ 10 mL K3-EDTA (CC6)

3. Processing of sample done? ☐ 1 Yes ☐ 0 No 3.1 If yes, time (24 hr clock) ☐☐ hr ☐☐ min

If no, or if delay between collection and centrifugation > 1 hour, complete the PROTOCOL DEVIATION SECTION

4. Specimen frozen? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION. If yes:

4.1 Date of freezing ☐☐ ☐☐☐ ☐☐☐☐ 4.2 Time (24 hr clock) ☐☐ hr ☐☐ min

If delay between collection and freezing > 2 hours, complete the PROTOCOL DEVIATION SECTION

Reminder concerning Blood Specimen Tracking: Information above on specimen collection and storage needs to be included in the specimen log (Excel file)

BABY'S FATHER SECTION

If the father has signed the consent form, you are allowed to ask these questions; if the father is not present, give the consent form to the woman to take home. You will be able to complete this section in Questionnaire 2. If the father is present, but refuses to sign the consent form, complete the PROTOCOL DEVIATION SECTION.

1. What is the year of birth of the baby's father? *yyyy* ☐☐☐☐

2. Highest education level achieved

☐ 0 Grade 8 or less ☐ 1 Some High School ☐ 2 High School Diploma
☐ 3 Some College Classes ☐ 4 College Diploma ☐ 5 Trade School Diploma
☐ 6 Undergraduate University Degree ☐ 7 Graduate University Degree (MSc, PhD)

3. People in Canada come from many racial or cultural groups. The baby's father may belong to more than one group on the following list. Is he… (check all that apply)

3.1 White ☐ 3.2 Chinese ☐ 3.3 South Asian (East Indian, Sri Lankan, Pakistani, etc.) ☐
3.4 Black ☐ 3.5 Filipino ☐ 3.6 Southeast Asian (Vietnamese, Cambodian, Malaysian, Laotian, etc.) ☐
3.7 Latin American ☐ 3.8 Arab ☐ 3.9 West Asian (Iranian, Afghan, etc.) ☐
3.10 Japanese ☐ 3.11 Korean ☐ 3.12 Aboriginal (North American Indian, Métis, or Inuit [Eskimo]) ☐
3.13 Other ☐ 3.13.1 If other, specify _____ 3.14 Refuse to answer ☐ 3.15 Don't know ☐

4. In what country was the baby's father born?

☐ 1 Canada ☐ 2 United States ☐ 3 Mexico ☐ 4 China ☐ 98 Don't know
☐ 5 Other 4.1 If other, specify _____

5. Did the baby's father have any treatment for infertility for this pregnancy? ☐ 1 Yes ☐ 0 No

5.1 If *yes*, specify _____

6. Is the baby's father employed? (please include any paid work, regardless of the number of hours worked)

☐ 1 Yes ☐ 0 No

If *yes*, for any jobs that the baby's father held during this pregnancy, please tell me the type of business and what type of work he does.

6.1 Job 1 ☐ 0 Part time ☐ 1 Full time
6.1.1 Type of business _____ 6.1.2 type of work _____

6.2 Job 2 ☐ 0 Part time ☐ 1 Full time
6.2.1 Type of business _____ 6.2.2 type of work _____

6.3 Job 3 ☐ 0 Part time ☐ 1 Full time
6.3.1 Type of business _____ 6.3.2 type of work _____

7. Does the baby's father smoke?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

7.1 If *yes*, does the baby's father regularly smoke in the home, that is, every day or almost every day?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

PREPARATION FOR VISIT 2 SECTION

1. Nutrient supplements form for the next visit given (Questionnaire 1B)? ☐ 1 Yes ☐ 0 No
   If no, complete the PROTOCOL DEVIATION SECTION
2. Appointment for the next visit is scheduled? ☐ 1 Yes ☐ 0 No

PROTOCOL DEVIATION SECTION

1. Were there any protocol deviations during this study visit? ☐ 1 Yes ☐ 0 No
   If *yes*, specify:

Study visit			Specify Reason
1.1	☐	Visit done outside the study window ($6^{0/7}$ to $12^{6/7}$)	1.1.1 _____
1.2	☐	Patient was not eligible after inclusion	1.2.1 _____
1.3	☐	A part of the questionnaire was not completed	1.3.1 _____
1.4	☐	Nutrient supplements form (1B) not given	1.4.1 _____
Anthropometric measurements			
1.5	☐	Blood pressure measurement not done	1.5.1 _____
1.6	☐	Weight not measured at this visit	1.6.1 _____
1.7	☐	Height not measured at this visit	1.7.1 _____
Specimen collection			
1.8	☐	Blood tube not collected	1.8.1 _____
1.9	☐	Blood tube lost	1.9.1 _____
1.10	☐	Blood processing problem	1.10.1 _____
1.11	☐	Blood freezing problem	1.11.1 _____
1.12	☐	Urine protein test by dipstick not done	1.12.1 _____
1.13	☐	24-hour urine protein test not done	1.13.1 _____
1.14	☐	Problem with urine sample collection	1.14.1 _____
1.15	☐	Urine sample freezing problem	1.15.1 _____
Baby's father information			
1.16	☐	1.16.1 Specify _____	
Other			
1.17	☐	1.17.1 Specify _____	

INTERVIEWER SECTION

1. Interviewer Initials ☐☐☐ 2. Duration of interview ☐☐☐ min
3. Signature _____ 4. Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

Reminder: Information regarding today's visit needs to be registered in the Follow-up log (Excel file)

UNUSED ALIQUOT LABELS

☐ CHECK HERE IF ALL LABELS HAVE BEEN USED FOR THIS VISIT

If NOT, stick the unused label(s) into the corresponding box(es):

Urine

CC1 (125ml Nalgene)

Label code **1**	Label code **2**
Label code **3**	Label code **4**
Label code **5**	Label code **6**
Label code **7**	Label code **8**

Maternal blood

CC2 (10 mL K2-EDTA)

Label code **9**	Label code **10**
Label code **11**	Label code **12**

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

UNUSED ALIQUOT LABELS (CONTINUED)

Maternal blood (continued)

CC3 (10 mL K2-EDTA)	Label code **13**	
CC4 (6 mL K2-EDTA)	Label code **16**	
CC5 (6 mL blue tube)	Label code **18**	Label code **19**
	Label code **20**	Label code **21**

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

UNUSED ALIQUOT LABELS (CONTINUED)

Maternal blood (continued)

	Label code
CC6 (10 mL K3-EDTA)	**24**

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 1B – Visit 1 – Nutrient Supplements Form

Center ☐☐ ID ☐☐☐ Monogram ☐☐☐

NUTRIENT SUPPLEMENTS FORM SECTION 1

Please complete this questionnaire around the 16th week of your pregnancy, before visit 2, then return it to research nurse at the next visit. Section 1 concerns your use during the last 24 hours (since this time yesterday). Section 2 concerns your use during the last 30 days (last month).

Complete the table by indicating the drug identification number (or DIN, NPN, NPN-HM), how much each supplement was taken at each occasion and how often each supplement was taken. Please use the example to complete the rest.

Important: some products do not have a drug identification number. In this case, check "No ID on bottle". Do not write down the lot number or the date of expiration.

Thank you for your participation.

1. Today's date (dd mmm yyyy) ☐☐ ☐☐☐ ☐☐☐☐

2. Yesterday (during last 24 hours), did you take vitamins, minerals, homeopathic medicines and/or natural products?

☐ Yes ☐ No

If yes, complete the tables below for each supplement. If no, go to NUTRIENT SUPPLEMENTS FORM SECTION 2 (page 3).

Example:

A. Name and description of product: MATERNA CENTRUM – PRENATAL MULTIVITAMIN

B. **Drug identification number on bottle (DIN / NPN / NPN-HM):** 123456789 ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ___ Capsule(s) 1 Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐ As needed ☒ 1x/day ☐ 2x/day
☐ 3x/day ☐ 4x/day
☐ Other If other, specify: _____

1 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐ As needed ☐ 1x/day ☐ 2x/day
☐ 3x/day ☐ 4x/day
☐ Other If other, specify: _____

2 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐ As needed ☐ 1x/day ☐ 2x/day
☐ 3x/day ☐ 4x/day
☐ Other If other, specify: _____

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 1B – Visit 1 – Nutrient Supplements Form

Center | ID | Monogram

3 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐ As needed ☐ 1x/day ☐ 2x/day
☐ 3x/day ☐ 4x/day
☐ Other If other, specifiy: _____

4 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐ As needed ☐ 1x/day ☐ 2x/day
☐ 3x/day ☐ 4x/day
☐ Other If other, specifiy: _____

5 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐ As needed ☐ 1x/day ☐ 2x/day
☐ 3x/day ☐ 4x/day
☐ Other If other, specifiy: _____

6 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐ As needed ☐ 1x/day ☐ 2x/day
☐ 3x/day ☐ 4x/day
☐ Other If other, specifiy: _____

7 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐ As needed ☐ 1x/day ☐ 2x/day
☐ 3x/day ☐ 4x/day
☐ Other If other, specifiy: _____

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 1B – Visit 1 – Nutrient Supplements Form

Center ☐☐ ID ☐☐☐ Monogram ☐☐☐

NUTRIENT SUPPLEMENTS FORM SECTION 2

1. During the last month (last 30 days), did you take vitamins, minerals, homeopathic medicines and/or natural products?

☐ Yes ☐ No

If yes, complete the tables below for each supplement.

Example:

A. Name and description of product : Vitamin C Natural Factors Liquid

B. **Drug identification number on bottle (DIN / NPN / NPN-HM):** 123456789 ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☒ 2 Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

0 2 times / ☒ day ☐ week ☐ month

---

1 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

---

2 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

---

3 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 1B – Visit 1 – Nutrient Supplements Form

Center ☐☐ ID ☐☐☐ Monogram ☐☐☐

4 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

5 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

6 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

7 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

8 **A. Name and description of product :**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 2 – Visit 2 – Follow-Up Visit *(between 16 0/7 and 21 6/7 weeks)*

Center | ID | Monogram | Day | Month | Year

VISIT STATUS SECTION

1. Visit done? ☐ 1 Yes ☐ 0 No If no, you must complete this section as well as NUTRIENT SUPPLEMENTS FORM SECTION & PREGNANCY STATUS SECTION

If no, specify:

1.1 Unable to schedule visit: ☐ 1 Yes ☐ 0 No

If yes, specify reason: 1.1.1 No answer ☐

1.1.2 Telephone disconnected ☐

1.1.3 The woman is too busy ☐

1.1.4 The woman is ill ☐

1.1.5 Other ☐ 1.1.5.1 Specify: _____

1.2 The woman refuses to participate further ☐ 1 Yes ☐ 0 No

1.3 The woman does not want to be contacted by letter or telephone ☐ 1 Yes ☐ 0 No

1.4 The woman refuses access to her medical or hospital records ☐ 1 Yes ☐ 0 No

1.5 The woman is no longer pregnant ☐ 1 Yes ☐ 0 No

1.6 Other 1.6.1 If other, specify: _____ ☐ 1 Yes ☐ 0 No

NUTRIENT SUPPLEMENTS FORM SECTION

1. Questionnaire 1B: ☐ 1 Returned ☐ 0 Not returned If not returned, complete the PROTOCOL DEVIATION SECTION

PREGNANCY STATUS SECTION

1. Is the woman still pregnant? ☐ 1 Yes ☐ 0 No ☐ 99 Information not available

If no 1.1 Specify the outcome:

☐ 0 Delivery of a live birth ☐ 1 Stillbirth (≥ 20 weeks gestation) ☐ 2 Spontaneous abortion

☐ 3 Therapeutic abortion ☐ 4 Hydatiform mole ☐ 5 Ectopic pregnancy

1.2 Specify cause _____ (if unknown, state "unknown")

1.3 Gestational age ☐☐ Weeks ☐ Days/7 ☐ 97 Unknown

1.4 Number of fetuses ☐ ☐ 97 Unknown

1.5 Gender (check all that apply) 1.5.1 Male ☐ 1.5.2 Female ☐ 1.5.3 Unknown ☐

IF PREGNANCY TERMINATED < 20 WEEKS:

2. Was the woman seen by a doctor at the delivery hospital? ☐ 1 Yes ☐ 0 No If yes, you need to wait for clinical report in woman's chart to complete this section.

If yes 2.1 Was there evidence of congenital anomalies? ☐ 1 Yes ☐ 0 No

If yes (check all that apply) 2.1.1 Detected by ultrasound ☐ 2.1.1.1 Diagnosis _____

2.1.2 Clinical assessment ☐ 2.1.2.1 Diagnosis _____

2.1.3 Confirmed by pathology ☐ 2.1.2.1 Diagnosis _____

If the woman is no longer pregnant: A) if delivery of a live-birth or pregnancy termination ≥ 20 weeks, you need to complete this questionnaire as well as questionnaires *4, 5a-5b, and 7*, and questionnaires *8a-8b* in the case of a *multiple pregnancy*;
B) if pregnancy terminated < 20 weeks, stop data collection here and complete questionnaire 7.

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 2 – Visit 2 – Follow-Up Visit *(between 16 0/7 and 21 6/7 weeks)*

Center ☐☐ ID ☐☐☐ Monogram ☐☐☐

GESTATIONAL AGE SECTION

1. At Visit 1, was there an ultrasound (US) report available with a dating based on crown-rump length (CRL)? ☐ Yes ☐ No

If Yes, go to EVOLUTION OF CURRENT PREGNANCY SECTION

If No, specify:

1.1 Was an ultrasound examination performed between Visit 1 and Visit 2? ☐ Yes ☐ No

If No, go to EVOLUTION OF CURRENT PREGNANCY SECTION

If Yes:

1.2 Is there any US report available for this woman? ☐ Yes ☐ No If No, complete the PROTOCOL DEVIATION SECTION and go to EVOLUTION OF CURRENT PREGNANCY SECTION

If Yes, complete the rest of the section:

Important: if there are more than one US report available, select in priority:

1) the earliest report with a dating based on CRL
2) if no CRL dating, the earliest report with a dating based on biparietal diameter (BPD) and femur length (FL)
3) if none of the above, the report with a dating based on sack

1.2.1 Date of the US selected *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

1.2.2 Gestational age (GA) written in the report ☐☐ Weeks ☐ Days/7

1.2.3 Measure(s) used for this GA (check all that apply): ☐ CRL ☐ BPD and FL ☐ Sack

1.2.4 Number of fetuses ☐ Don't know ☐

1.2.5 Define the accurate GA at visit 2: if US and LMP dates differ by ≤ 7 days, base GA on LMP date; if they differ by > 7 days, use ultrasound result (*see questionnaire 1 for LMP date*)

☐☐ Weeks ☐ Days/7

1.2.6 GA at visit 2 estimated by: ☐ Last Menstrual Period **or** ☐ Ultrasound

1.2.7 Expected date of confinement *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

EVOLUTION OF CURRENT PREGNANCY SECTION

1. Since the last visit, have you had any complications with this pregnancy? ☐ Yes ☐ No

1.1 If *yes*, what complications have you had with this pregnancy?

☐ Repeated vomiting with weight loss ☐ Vaginal Bleeding

☐ Other 1.1.1 If other, specify _____

2. Have you had any acute medical conditions during this pregnancy? ☐ Yes ☐ No

(A condition of rapid onset, severe symptoms and brief duration)

If *yes*: Medical condition Treatment *(more than 1 treatment possible per line)*

2.1 _____ 2.1.1 _____

2.2 _____ 2.2.1 _____

2.3 _____ 2.3.1 _____

2.4 _____ 2.4.1 _____

2.5 _____ 2.5.1 _____

2.6 _____ 2.6.1 _____

CURRENT MEDICATION SECTION

1. Are you taking any medication? Ask for any current medication you didn't record in the previous section (ASA, antihypertensives, insulin, hypoglycemics, etc.).

☐ 0 **None** If none, go to ANTHROPOMETRIC MEASUREMENT SECTION

A. Name of the product (When possible, use generic name, except for combination products)	B. Indication
1.1A	1.1B
1.2A	1.2B
1.3A	1.3B
1.4A	1.4B
1.5A	1.5B
1.6A	1.6B
1.7A	1.7B
1.8A	1.8B
1.9A	1.9B
1.10A	1.10B

ANTHROPOMETRIC MEASUREMENT SECTION

1. Weight measured at this visit ☐☐☐ • ☐ ☐ 0 Kg ☐ 1 Pounds
If not done, complete the PROTOCOL DEVIATION SECTION

BLOOD PRESSURE AND URINE COLLECTION SECTION

BLOOD PRESSURE MEASUREMENT DURING THIS VISIT (SITTING, LEFT ARM)

1. Have blood pressure measurements been taken? ☐ 1 Yes ☐ 0 No
If no, complete the PROTOCOL DEVIATION SECTION

If yes, record 1.1 1st Measure ☐☐☐ / ☐☐☐ mm/Hg
systolic *diastolic*

One minute apart:

1.2 2nd Measure ☐☐☐ / ☐☐☐ mm/Hg
systolic *diastolic*

BLOOD PRESSURE AND URINE COLLECTION SECTION (CONTINUED)

URINE SAMPLE COLLECTION

Collect a minimum of 55 ml of urine into a 125ml Nalgene container

2. Collection of sample done (CC7) ☐ 1 Yes ☐ 0 No If no or less than expected, complete the PROTOCOL DEVIATION SECTION; if *yes:*

2.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 2.2 Time (24 hr clock) ☐☐ hr ☐☐ min

2.3 Number of hours since last urination (prior to this one) ☐☐ hr ☐☐ min

2.4 Processing and freezing of urine sample done ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If *yes* 2.4.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 2.4.2 Time (24 hr clock) ☐☐ hr ☐☐ min

URINE PROTEIN DIPSTICK TEST Transfer at least 5ml of urine from the 125ml Nalgene container into a 60ml urine container

3. Urine protein test by dipstick done ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

3.1 If *yes*, indicate the result obtained

☐ Zero ☐ Trace ☐ 1+ or 30 mg/dL or 0.3 g/L ☐ 2+ or 100 mg/dL or 1.0 g/L ☐ 3+ or 300 mg/dL or 3.0 g/L ☐ 4+ or 2000 mg/dL or 20 g/L

If protein on dipstick ≥ 1+, check in the patient chart if a 24-hour urine protein test has been ordered in the last seven days:

3.1.1 Was the 24-hour urine test ordered because of a positive dipstick? If not, inform to the patient's doctor the dipstick results and indicate that documentation of a 24-hour urine protein test is highly desirable for the study protocol. If he orders it, mark Yes. If not, specify the reason in the PROTOCOL DEVIATION SECTION.

☐ 1 Yes ☐ 0 No

If *yes*, probe: If the 24-hour urine protein test was ordered today, explain to the patient it should be done within the next 7 days and wait for the results to complete the rest of the section.

3.1.1.1 Indicate the result obtained ☐☐☐☐☐ mg/24 h **or** ☐☐ • ☐ g/L

3.1.1.2 Date test performed *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

BLOOD SAMPLE COLLECTION SECTION

1. Was the woman fasting? ☐ 1 Yes ☐ 0 No

2. Specimen collection done? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

2.1 If *yes* Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐ 2.2 Time (24 hr clock) ☐☐ hr ☐☐ min

2.3 Check tubes collected (3 x10 ml and 2 X 6 ml expected) If less specimens than expected, complete the PROTOCOL DEVIATION SECTION

☐ 10 mL K2-EDTA (CC8) ☐ 10 mL K2-EDTA (CC9) ☐ 6 mL K2-EDTA (CC10) ☐ 6 mL K2-EDTA (CC11) ☐ 10 mL K3-EDTA (CC12)

3. Processing of sample done? ☐ 1 Yes ☐ 0 No 3.1 If yes, time (24 hr clock) ☐☐ hr ☐☐ min

If no, or if delay between collection and centrifugation > 1 hour, complete the PROTOCOL DEVIATION SECTION

4. Specimen frozen? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION. If yes:

4.1 Date of freezing ☐☐ ☐☐☐ ☐☐☐☐ *ddmmmyyyy* 4.2 Time ☐☐ hr ☐☐ min

If delay between collection and freezing > 2 hours, complete the PROTOCOL DEVIATION SECTION

Reminder concerning Blood Specimen Tracking: Information above on specimen collection and storage needs to be included in the specimen log (Excel file)

CLINICAL TESTS SECTION If they are available on the woman's chart. If more than one test was done, enter results of 1st test. *Please, pay particular attention to units.*

1. Hemoglobin: Done ☐ 1 Yes ☐ 0 No If yes 1.1 Result ☐☐☐ g/L **or** ☐☐•☐ g/dl
1.2 Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐

2. AFP: Done ☐ 1 Yes ☐ 0 No If yes 2.1 Result ☐☐☐☐ ng/mL **or** ☐☐☐☐ µg/L
2.2 Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐

3. HCG: Done ☐ 1 Yes ☐ 0 No If yes 3.1 Result ☐☐☐☐☐☐ IU/L
3.2 Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐

4. Estriol: Done ☐ 1 Yes ☐ 0 No If yes 4.1 Result ☐☐☐☐ ng/mL **or** ☐☐☐☐ pmol/L
4.2 Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐

5. C-reactive protein: Done ☐ 1 Yes ☐ 0 No If yes 5.1 Result ☐☐ mg/L
5.2 Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐

6. T_4: Done ☐ 1 Yes ☐ 0 No If yes 6.1 Result ☐☐☐ mg/L **or** ☐☐☐ nmol/L
6.2 Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐

7. Free T_4: Done ☐ 1 Yes ☐ 0 No If yes 7.1 Result ☐☐☐ ng/L **or** ☐☐☐ pmol/L
7.2 Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐

8. T_3: Done ☐ 1 Yes ☐ 0 No If yes 8.1 Result ☐☐☐ mg/L **or** ☐☐☐ nmol/L
8.2 Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐

9. TSH: Done ☐ 1 Yes ☐ 0 No If yes 9.1 Result ☐☐•☐ mIU/L
9.2 Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐

10. Serum ferritin: Done ☐ 1 Yes ☐ 0 No If yes 10.1 Result ☐☐☐☐ ng/mL
10.2 Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐

Center ☐☐ ID ☐☐☐ Monogram ☐☐☐

BABY'S FATHER SECTION

1. Was this section completed in questionnaire 1? ☐ 1 Yes ☐ 0 No If yes, go to FOOD FREQUENCY QUESTIONNAIRE SECTION
If no, and the woman returned the consent form signed by the father, you are allowed to ask these questions;
If no, and the woman did not return the consent form signed by the father, complete the PROTOCOL DEVIATION SECTION.

2. What is the year of birth of the baby's father? *yyyy* ☐☐☐☐

3. Highest education level achieved

☐ 0 Grade 8 or less ☐ 1 Some High School ☐ 2 High School Diploma
☐ 3 Some College Classes ☐ 4 College Diploma ☐ 5 Trade School Diploma
☐ 6 Undergraduate University Degree ☐ 7 Graduate University Degree (MSc, PhD)

4. People in Canada come from many racial or cultural groups. The baby's father may belong to more than one group on the following list. Is he… (check all that apply)

4.1 White ☐ 4.2 Chinese ☐ 4.3 South Asian (East Indian, Sri Lankan, Pakistani, etc.) ☐
4.4 Black ☐ 4.5 Filipino ☐ 4.6 Southeast Asian (Vietnamese, Cambodian, Malaysian, Laotian, etc.) ☐
4.7 Latin American ☐ 4.8 Arab ☐ 4.9 West Asian (Iranian, Afghan, etc.) ☐
4.10 Japanese ☐ 4.11 Korean ☐ 4.12 Aboriginal (North American Indian, Métis, or Inuit [Eskimo]) ☐
4.13 Other ☐ 4.13.1 If other, specify _____ 4.14 Refuse to answer ☐ 4.15 Don't know ☐

5. In what country was the baby's father born?

☐ 1 Canada ☐ 2 United States ☐ 3 Mexico ☐ 4 China ☐ 98 Don't know
☐ 5 Other 5.1 If other, specify _____

6. Did the baby's father have any treatment for infertility for this pregnancy? ☐ 1 Yes ☐ 0 No

6.1 If *yes*, specify _____

7. Is the baby's father employed? (please include any paid work, regardless of the number of hours worked)

☐ 1 Yes ☐ 0 No

If *yes*, for any jobs that the baby's father held during this pregnancy, please tell me the type of business and what type of work he does.

7.1 Job 1 ☐ 0 Part time ☐ 1 Full time
7.1.1 Type of business _____ 7.1.2 type of work _____

7.2 Job 2 ☐ 0 Part time ☐ 1 Full time
7.2.1 Type of business _____ 7.2.2 type of work _____

7.3 Job 3 ☐ 0 Part time ☐ 1 Full time
7.3.1 Type of business _____ 7.3.2 type of work _____

8. Does the baby's father smoke?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

8.1 If *yes*, does the baby's father regularly smoke in the home, that is, every day or almost every day?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

FOOD FREQUENCY QUESTIONNAIRE SECTION

In the next part of the interview, we are interested in knowing whether you ate certain foods over the last month. If you did eat them, we would like to know how often you ate them. We are interested only in whether you have eaten them in the last month. So, if you have not eaten those foods at least once within the past month (that is since..... give the date of 1 month ago), they are not important to this part of the interview.
"Please think about the last 4 weeks. Were they work weeks or holidays times? Did they involve a special occasion such as a wedding or party?"

"Now I am going to read the list of foods"

"In the past month did you consume_____?"

IF NO, MOVE TO NEXT FOOD.

IF YES, THEN GO TO THE NEXT COLUMN "B. FREQUENCY" and THEN, ASK SERVING SIZE IN COLUMN "C"

A. FOOD		B. FREQUENCY	C. SERVING SIZE	
In the past month, did you consume:		How often did you consume it per day, week or month?	Using the average serving size as a guide, would your usual serving size be:	**Average Serving**
Vegetables				
1. Broccoli	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
2. Spinach - raw	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
3. Spinach - cooked	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
4. Sweet peppers *(any colours, fresh or processed)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup or ½ pepper
5. Tomatoes *(fresh or canned, incl. tomato based sauce)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 tomato or 125 ml or ½ cup
6. Tomato or vegetable juice	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
7. Potatoes with the skin *(skin eaten)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 medium potato
Fruits				
8. Citrus fruits – excl. juices	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 orange or ½ grapefruit

FOOD FREQUENCY QUESTIONNAIRE SECTION (continued)

A. FOOD		B. FREQUENCY	C. SERVING SIZE	
In the past month, did you consume:		How often did you consume it per day, week or month?	Using the average serving size as a guide, would your usual serving size be:	**Average Serving**
Fruits **(continued)**				
9. Orange juice with calcium	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
10. Other fruit juices and drinks	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
Meat, Poultry, Fish and Alternatives				
11. Red meat *(beef, hamburger, pork, lamb)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz or 1 patty
12. Poultry *(chicken, turkey)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz
13. Wild game – animals	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz
14. Wild game – birds	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz
15. Liver	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz
16. Luncheon meats *(cold cuts)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	3 slices
17. Sausages or wieners *(with or without a bun)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 sausage
18. Bacon	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	3 slices
19. Eggs and egg dishes	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	2 eggs
20. Fish – excl. shellfish *(fish sticks, tuna, salmon, trout, etc.)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz
21. Clams and oysters	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	6 clams or oysters

FOOD FREQUENCY QUESTIONNAIRE SECTION (continued)

A. FOOD		B. FREQUENCY	C. SERVING SIZE	
In the past month, did you consume:		How often did you consume it per day, week or month?	Using the average serving size as a guide, would your usual serving size be:	**Average Serving**
Meat, Poultry, Fish and Alternatives **(continued)**				
22. Other shellfish *(shrimp, lobster, crab, etc.)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	10 medium shrimp or 75 g or 2.5 oz
23. Beans and dry peas *(baked beans, pea soup, chilli, hummus, lentils, etc.)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
Milk Products				
24. Milk (*whole, 2%, 1%, skim, chocolate, Lactaid)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
25. Soy and rice beverages fortified with calcium and vitamin D	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
26. Hard cheese *(parmesan, romano)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	30 ml or 2 tablespoons shredded
27. Cheese – Regular, low fat or partly skimmed *(e.g. mozzarella and cheddar)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	30 g or 1 oz
28. Processed cheese *(cheese slices or spread)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 slice or 2 tablespoons
29. Cottage cheese	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
30. Cream cheese	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	30 ml or 2 tablespoons
31. Yogurt – all types	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
32. Ice cream, frozen yogurt, ice milk or milkshakes	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
Grain Products				
33. Cereal – cold	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup

FOOD FREQUENCY QUESTIONNAIRE SECTION (continued)

A. FOOD		B. FREQUENCY	C. SERVING SIZE	
In the past month, did you consume:		How often did you consume it per day, week or month?	Using the average serving size as a guide, would your usual serving size be:	**Average Serving**
Grain Products **(continued)**				
34. Cereal – hot	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	175 ml or ¾ cup
35. White bread, rolls, buns, bagels, pita bread or tortillas	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	2 slices, rolls, buns, tortillas 1 bagel, pita
36. Whole grain bread *(whole wheat, oatmeal, rye, pumpernickel, etc.)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	2 slices
37. Pasta	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
38. Crackers	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	4 crackers
39. Cookies	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	2 cookies
40. Cake, muffins, pies, pastries, doughnuts	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 piece, muffin, pastry or doughnut
41. Cereal, muffin & oatmeal bars *(Nutri-Grain®, Hop & Go®, Oatmeal-To-Go®, etc.)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 bar
Other Foods				
42. Pizza	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	2 slices
43. Tea	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
44. Coffee	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
45. Margarine	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	5 ml or 1 teaspoon
46. Butter	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	5 ml or 1 teaspoon

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 2 – Visit 2 – Follow-Up Visit *(between 16 0/7 and 21 6/7 weeks)*

Center ☐☐ ID ☐☐☐ Monogram ☐☐☐

PROTOCOL DEVIATION SECTION

1. Were there any protocol deviations during this study visit? ☐ 1 Yes ☐ 0 No

If yes, specify:

		Study visit	**Specify Reason**
1.1	☐	Study visit done outside the visit window (16 0/7 to 21 6/7)	1.1.1 _____
1.2	☐	A part of the questionnaire was not completed	1.2.1 _____
1.3	☐	Questionnaire 1B on nutrient supplements not returned	1.3.1 _____
		Anthropometric measurement	
1.4	☐	Blood pressure measurement not done	1.4.1 _____
1.5	☐	Weight not measured at this visit	1.5.1 _____
		Specimen collection	
1.6	☐	Blood tube not collected	1.6.1 _____
1.7	☐	Blood tube lost	1.7.1 _____
1.8	☐	Blood processing problem	1.8.1 _____
1.9	☐	Blood freezing problem	1.9.1 _____
1.10	☐	Urine protein test by dipstick not done	1.10.1 _____
1.11	☐	24-hour urine protein test not done	1.11.1 _____
1.12	☐	Problem with urine sample collection	1.12.1 _____
1.13	☐	Urine sample freezing problem	1.13.1 _____
		Ultrasound reports	
1.14	☐	Report of ultrasound not available	1.14.1 _____
		Food frequency questionnaire	
1.15	☐	A part of the questionnaire not completed	1.15.1 _____
		Baby's father information	
1.16	☐	1.16.1 Specify _____	
		Other	
1.17	☐	1.17.1 Specify _____	
1.18	☐	1.18.1 Specify _____	

APPOINTMENT FOR THE NEXT VISIT SECTION

1. The next visit is scheduled ☐ 1 Yes ☐ 0 No

INTERVIEWER SECTION

1. Interviewer Initials ☐☐☐ 2. Duration of interview ☐☐☐ min

3. Signature _____ 4. Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

Reminder: Information regarding today's visit needs to be registered in the Follow-up log (Excel file)

UNUSED ALIQUOT LABELS

☐ CHECK HERE IF ALL LABELS HAVE BEEN USED FOR THIS VISIT

If NOT, stick the unused label(s) into the corresponding box(es):

Urine

CC7 (125ml Nalgene)

Label code	Label code
29	**30**

Maternal blood

CC8 (10 mL K2-EDTA)

Label code	Label code
32	**33**

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

UNUSED ALIQUOT LABELS (CONTINUED)

Maternal blood (continued)

CC9 (10 mL K2-EDTA)		
CC10 (6 mL K2-EDTA)	Label code **37**	
	Label code **38**	Label code **39**
CC11 (6 mL K2-EDTA)	Label code **40**	Label code **41**
CC12 (10 mL K3-EDTA)	Label code **44**	

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 3 – Visit 3 – Follow-up visit *(between 32 0/7 and 34 6/7 weeks)*

Center | ID | Monogram | Day | Month | Year

I want to start by thanking you for your help with this study. I will be asking you questions about your home, your diet during pregnancy, chemicals you might have been exposed to and drugs or medication you might have taken during your pregnancy. If a question makes you feel uncomfortable you don't have to answer it. We would appreciate you being as honest as possible in your answers. Do you have any questions before we begin?

VISIT STATUS SECTION

1. Visit done? ☐ 1Yes ☐ 0No If no, you must complete this section as well as PREGNANCY STATUS SECTION

If no, specify:

1.1 Unable to schedule visit: ☐ 1Yes ☐ 0No

If yes, specify reason: 1.1.1 No answer ☐

1.1.2 Telephone disconnected ☐

1.1.3 The woman is too busy ☐

1.1.4 The woman is ill ☐

1.1.5 Other ☐ 1.1.5.1 Specify: _____

1.2 The woman refuses to participate further ☐ 1Yes ☐ 0No

1.3 The woman does not want to be contacted by letter or telephone ☐ 1Yes ☐ 0No

1.4 The woman refuses access to her medical or hospital records ☐ 1Yes ☐ 0No

1.5 The woman is no longer pregnant ☐ 1Yes ☐ 0No

1.6 Other ☐ 1Yes ☐ 0No

If other 1.6.1 Specify: _____

PREGNANCY STATUS SECTION

1. Is the woman still pregnant? ☐ 1Yes ☐ 0No ☐ 99Information not available

If no 1.1 Specify the outcome:

☐ 0Therapeutic termination ☐ 1Stillbirth ☐ 2Delivery of a live-birth

If the woman is no longer pregnant: you need to complete this questionnaire as well as questionnaires *4, 5a-5b, and 7*. You also need to complete questionnaires *6a-6b-6c* if the woman is able to participate in milk collection, and questionnaires *8a-8b* in the case of a *multiple pregnancy*.

EVOLUTION OF CURRENT PREGNANCY SECTION

1. Since the last visit, have you had any complications with this pregnancy? ☐ 1 Yes ☐ 0 No
   1.1 If *yes*, what complications have you had with this pregnancy?
   ☐ 0 Repeated vomiting with weight loss ☐ 1 Vaginal Bleeding
   ☐ 2 Other 1.1.1 If other, specify _____

2. Have you had any acute medical conditions during this pregnancy? ☐ 1 Yes ☐ 0 No
   (A condition of rapid onset, severe symptoms and brief duration)

If *yes*: Medical condition	Treatment *(more than 1 treatment possible per line)*
2.1 _____	2.1.1 _____
2.2 _____	2.2.1 _____
2.3 _____	2.3.1 _____
2.4 _____	2.4.1 _____
2.5 _____	2.5.1 _____
2.6 _____	2.6.1 _____

CURRENT MEDICATION SECTION

1. Are you taking any medication? Ask for any current medication you didn't record in the previous section (ASA, antihypertensives, insulin, hypoglycemics, etc).

☐ 0 **None** If none, go to EMPLOYMENT STATUS SECTION

A. Name of the product (When possible, use generic name, except for combination products)	**B. Indication**
1.1A	1.1B
1.2A	1.2B
1.3A	1.3B
1.4A	1.4B
1.5A	1.5B
1.6A	1.6B
1.7A	1.7B
1.8A	1.8B
1.9A	1.9B
1.10A	1.10B

EMPLOYMENT STATUS SECTION

1. During any part of your pregnancy, have you been employed *(Probe: had any type of job)* ☐ 1 Yes ☐ 0 No
   If No, go to ENVIRONMENTAL EXPOSURES SECTION - PART A
2. Has your employment status changed since your first trimester (visit 1) ☐ 1 Yes ☐ 0 No
   Probe: change of employer, type of work or hours of work

   If No, go to question 3.

   If *yes*,

   2.1 How has your employment status changed?

   ☐ 0 No longer employed (go to question 3)

   ☐ 1 Change of employer, type of work, or hours of work (specify below)

   2.1.1 Job 1 ☐ 0 Part time ☐ 1 Full time

   2.1.1.1 Type of business _____ 2.1.1.2 Type of work _____

   2.1.2 Job 2 ☐ 0 Part time ☐ 1 Full time

   2.1.2.1 Type of business _____ 2.1.2.2 Type of work _____

   2.1.3 Job 3 ☐ 0 Part time ☐ 1 Full time

   2.1.3.1 Type of business _____ 2.1.3.2 Type of work _____

3. During your pregnancy, have there been any non-routine work events, like chemical leaks or spills in your workplace?

   ☐ 1 Yes ☐ 0 No ☐ 98 Don't know

   3.1 If *yes*, what chemical spilled? _____

   3.2 Were you directly exposed to the chemical spill? ☐ 1 Yes ☐ 0 No ☐ 97 N/A ☐ 98 Don't know

4. Prior to or during your pregnancy, were you required or advised by any means to wear protective equipment such as clothing or a respirator (please specify the type of equipment and what it is supposed to protect you from)?

   4.1 Prior to pregnancy ☐ 1 Yes ☐ 0 No ☐ 97 N/A ☐ 98 Don't know

   4.1.1 If *yes*, specify type _____ 4.1.2 Specify what _____

   4.1.3 How long ago? ☐ 0 <3 months ☐ 1 3-12 months ☐ 2 >12 months ☐ 98 Don't know

   4.2 During pregnancy ☐ 1 Yes ☐ 0 No ☐ 97 N/A ☐ 98 Don't know

   4.2.1 If *yes*, specify type _____ 4.2.2 Specify what _____

5. During your pregnancy, how often do you wear protective equipment on the job? Would you say?

   ☐ 0 Always ☐ 1 2-3 times / week ☐ 2 1/week ☐ 3 1/month

   ☐ 4 Never ☐ 97 N/A ☐ 98 Don't know

ENVIRONMENTAL EXPOSURES SECTION - PART A

*Now I am going to ask you about other environmental exposures during your pregnancy. Please think about the **current pregnancy, SINCE VISIT 1**, either at work or at home.*

A. Have you been exposed to [INSERT EXPOSURE FROM TABLE BELOW]? [RECORD ANSWER IN TABLE BELOW]

If Yes, ASK: ***If NO, go to next product***

B. Was your exposure direct? (Probe: Did you handle [INSERT EXPOSURE]?)
C. How often are you exposed to [INSERT EXPOSURE] ? Would you say. . .
D. Did the exposure to [INSERT EXPOSURE] occur while you were at work?
E. In what trimester(s) were you exposed to [INSERT EXPOSURE]? (Check more than one if applies)

EXPOSURE	A. Exposure during pregnancy	B. Direct Exposure Doing it yourself or direct contact with the substance	C. Frequency	D. Exposure at work	E. List trimester(s) you were exposed
1. Coal products from hot asphalt or tar roofing material	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
2. Carbon black from copying or printing machines	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
3. Clothing Dyes	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
4. Fresh oil-based paint	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
5. Fresh latex or acrylic paint	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
6. Fresh spray paint (in aerosol container)	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
7. Solvents	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
Cosmetic Products					
8. Hair dyes	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
9. Hair relaxers	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
10. Perm solutions	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
11. Hair sprays or gels	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know

ENVIRONMENTAL EXPOSURES SECTION - PART A (CONTINUED)

EXPOSURE	A. Exposure During Pregnancy	B. Direct Exposure Doing it yourself or direct contact with the substance	C. Frequency	D. Exposure at work	E. List trimester(s) you were exposed
12. Nail care products (for application or removal)	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
13. Makeup (for example lip liner, lipstick, foundation, mascara, blush, eyeliner, eye shadow)	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
14. Fragrances, perfumes or colognes	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
15. Skin lotions or moisturizers	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
Pesticides for:					
16. Lawn/garden weeds	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
17. Lawn/garden insects	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
18. House plant insects	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
19. Pet fleas	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know
20. Residential pests (cockroaches, rodents, ants, etc)	☐ Yes ☐ No ☐ Don't know	☐ Yes ☐ No ☐ Don't know	☐ Daily ☐ 2-3/week ☐ 1/ week ☐ 1/ month ☐ <1 month ☐ 1-2/pregnancy	☐ Yes ☐ No ☐ Don't know	☐ T1 ☐ T2 ☐ T3 ☐ Don't know

ENVIRONMENTAL EXPOSURES SECTION - PART B

1. Have any renovations been done in your home during the last 3 months?

☐ 1 Yes ☐ 0 No

If *yes*, check all that apply:

1.1 ☐ Carpets – installed new/replaced old
1.2 ☐ Commercial cleaning – drapes, carpets or furniture
1.3 ☐ Furniture – restoring/refinishing
1.4 ☐ Insulation – replaced, installed new or removed old
1.5 ☐ Landscaping
1.6 ☐ Paint – removed by scraping, sanding or heat gun
1.7 ☐ Paint – removed using chemical strippers
1.8 ☐ Painting – Exterior – with spray paint (in aerosol container)
1.9 ☐ Painting – Interior – with spray paint (in aerosol container)
1.10 ☐ Painting – Interior/Exterior – with oil-based (alkyd) paints
1.11 ☐ Painting – Interior/Exterior – with latex or acrylic paints
1.12 ☐ Vinyl Flooring – replaced
1.13 ☐ Wallpaper – removed/added
1.14 ☐ Windows – applied caulking, grout or sealant
1.15 ☐ Wood Flooring – refinishing
1.16 ☐ Other 1.16.1 If other, specify_____

2. Since visit 1, have you had any mercury-silver (also known as amalgam) dental fillings replaced?

☐ 1 Yes ☐ 0 No

3. Currently, how many mercury-silver dental fillings do you have?

☐ 0 0 ☐ 1 1-4 ☐ 2 5-9 ☐ 3 10-14 ☐ 4 15+ ☐ 98 Don't know

ENVIRONMENTAL EXPOSURES SECTION – PART B (CONTINUED)

4. The following question refers to activities or hobbies. Have you or anyone living in your home done any of the following in **the past 3 months**?

Activity/Hobby	A. Participant	B. Anyone living in home
4.1 Painting	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.2 Pottery or ceramics	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.3 Candle making	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.4 Stained glass soldering	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.5 Welding or soldering	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.6 Print making	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.7 Auto body repair	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.8 Developing of photographs (darkroom lab)	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.9 Furniture stripping/refinishing	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.10 Working with dyes, fibers and fabrics	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.11 Jewellery, hollowware and enamelling	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.12 Metal working	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.13 Electronics	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.14 Automotive mechanical work	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.15 Silk screening	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.16 Wood working	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.17 Crafts involving glues, solvents	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.18 Use of chemicals to control weeds in lawn or garden	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.19 Use of chemicals to control insects or disease in lawn, garden or interior house plants	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.20 Use of chemicals to control pests indoors (e.g., rodents, fleas)	☐ 1 Yes ☐ 0 No	☐ 1 Yes ☐ 0 No
4.21 Other 4.21.1 If other, specify	☐ 1 Yes ☐ 0 No _____	☐ 1 Yes ☐ 0 No _____

ENVIRONMENTAL EXPOSURES SECTION – PART B (CONTINUED)

5. Do you have or have you previously had a carpet or upholstery in your home or in your car that was treated with a non-stick or stain-resistant material? ☐ 1 Yes ☐ 0 No ☐ 98 Don't know

6. In the last month (30 days), have you used cooking vessels designed for stove and/or oven use that are made of any of the following material types?

6.1 Non stick	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.2 Cast iron	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.3 Glazed ceramic	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.4 Stainless steel	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.5 Aluminium	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.6 Other metallics (such as bronze or copper)	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
6.7 Other	☐ 1 Yes	☐ 0 No	☐ 98 Don't know

6.7.1 If other, specify _____

7. In the last month (30 days), have you used any of the following vessels for heating food or drinks in the microwave?

7.1 Non stick	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
7.2 Glazed ceramic	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
7.3 Plastic	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
7.4 Disposable food containers (e.g., margarine tubs)	☐ 1 Yes	☐ 0 No	☐ 98 Don't know
7.5 Other	☐ 1 Yes	☐ 0 No	☐ 98 Don't know

7.5.1 If other, specify _____

SMOKING SECTION - PART A

SMOKING HISTORY / ACTIVE SMOKER *Now I'd like to ask you about your smoking history. Please include cigarettes that are ready-made as well as cigarettes that you make yourself.*

1. Have you ever smoked at least 100 cigarettes over your lifetime (about 4 packs)?

☐ 1 Yes ☐ 0 No (if no, go to SMOKING SECTION - PART B)

2. At the present time, do you smoke cigarettes daily, occasionally or not at all?

☐ 0 Daily (GO TO QUESTION 5) ☐ 1 Occasionally (GO TO QUESTION 3) ☐ 2 Not at all (GO TO QUESTION 7)

3. On the days that you do smoke, how many cigarettes do you usually smoke?

☐☐ 0 Cigarettes ☐ 98 Don't know ☐ 99 Refused to answer

4. In the past month, on how many days have you smoked one or more cigarettes?

☐☐ 0 Days (GO TO QUESTION 6) ☐ 98 Don't know ☐ 99 Refused to answer

5. How many cigarettes do you smoke each day now?

☐☐ 0 Cigarettes ☐ 98 Don't know ☐ 99 Refused to answer

6.A What brand do you usually smoke? (*check only one – if more than one, check "No regular brand"*)

☐ 0 Belvedere Select (Extra Mild)
☐ 1 Number 7
☐ 2 Canadian Classics
☐ 3 Number 7 Blue (Light)
☐ 4 Canadian Classics Silver (Light)
☐ 5 Number 7 Silver (Extra Mild)
☐ 6 Canadian Classics White (Extra Light)
☐ 7 Peter Jackson Full Flavour
☐ 8 DuMaurier
☐ 9 Peter Jackson Select Flavour (Light)
☐ 10 DuMaurier Distinct (Light)
☐ 11 Peter Jackson Mellow Flavour (Extra Light)
☐ 12 DuMaurier Premiere (Extra Light)
☐ 13 Player's Original Flavour
☐ 14 DuMaurier Prestige (Ultra Light)
☐ 15 Player's Rich Flavour (Light)
☐ 16 Export "A" Medium
☐ 17 Player's Smooth Flavour (Extra Light)
☐ 18 Export "A" Smooth (Light)
☐ 19 Cigarettes from First Nations/Native Reserve
☐ 20 Export "A" Ultra Smooth (Ultra Light)
☐ 21 No regular brand (*check this if the patient smokes multiple brands)*
☐ 22 Matinée Slims (Extra Mild)
☐ 23 Other 6.A.1 If other, specify _____

6.B Specify the size:

☐ 0 King Size
☐ 1 Regular Size
☐ 2 100's
☐ 3 Other 6.B.1 If other, specify _____

SMOKING SECTION - PART A *(continued)*

SMOKING HISTORY / ACTIVE SMOKER *(continued)*

7. At what age did you smoke your first whole cigarette? ☐☐ Years

8. Have you stopped smoking? ☐ Yes ☐ No

8.1 If *yes*, when did you stop smoking?

☐ When I knew I was pregnant → 8.1.1 Date of cessation *dd mmm yyyy* ☐☐☐☐☐ ☐☐☐☐

☐ < 1 year ago ☐ ≥ 1 and < 2 years ago (go to SMOKING SECTION - PART B)

☐ ≥ 2 and < 3 years ago (go to SMOKING SECTION - PART B) ☐ ≥ 3 years ago (go to SMOKING SECTION - PART B)

☐ Don't know ☐ Refuse to answer

9. During the past 3 months, have you stopped smoking for at least 24 hours because you were trying to quit?

☐ Yes ☐ No ☐ Don't know ☐ Refuse to answer

9.1 If *yes*, how many times ☐☐ ☐ Don't know ☐ Refuse to answer

10. During the past 3 months, did you try any of the following to quit smoking?

10. 1 Nicotine patch	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10. 2 Nicorettes or other nicotine gum, lozenge or candy	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer
10. 3 Medication such as Zyban	☐ Yes	☐ No	☐ Don't know	☐ Refuse to answer

11. Does your doctor know that you [smoke/smoked] cigarettes?

☐ Yes ☐ No (go to SMOKING SECTION - PART B)

☐ Don't know (go to SMOKING SECTION - PART B) ☐ Refuse to answer (go to SMOKING SECTION - PART B)

12. During the past 3 months, did your doctor advise you to quit smoking?

☐ Yes ☐ No (go to SMOKING SECTION - PART B) ☐ Don't know ☐ Refuse to answer

13. During the past 3 months, did your doctor give you any specific help or information to quit smoking?

☐ Yes ☐ No (go to SMOKING SECTION - PART B) ☐ Don't know ☐ Refuse to answer

13.1 If *yes*, what type of help did the doctor give?

☐ Referral to a one-on-one cessation program ☐ Referral to a group cessation program

☐ Recommended use of nicotine patch or nicotine gum ☐ Recommended Zyban or other medication

☐ Provided self-help information (e.g., pamphlet, referral to Website) ☐ Own doctor offered counseling

☐ Other 13.1.1 If other, specify _____

SMOKING SECTION - PART B

EXPOSURE TO SECOND-HAND SMOKE

1. Including both household members and regular visitors, does anyone smoke inside your home, every day or almost every day? Note : Include cigarettes, cigars and pipes.
   ☐ 1 Yes ☐ 0 No (Go to question 4) ☐ 98 Don't know ☐ 99 Refuse to answer

2. How many people smoke inside your home every day or almost every day? Note: Include household members and regular visitors
   ☐☐ 0 People

3. On a typical day, how many cigarettes are smoked inside your home?
   ☐ 0 1 to 10 ☐ 1 11 to 20 ☐ 2 21 to 30 ☐ 3 31 to 40 ☐ 4 41 or more
   ☐ 98 Don't know ☐ 99 Refuse to answer

4. In the past month, were you exposed to second-hand smoke, every day or almost every day, in a car or other private vehicle?
   ☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

5. During your pregnancy, has anyone in your workplace smoked in your presence? (including breaks, lunch)
   ☐ 1 Yes ☐ 0 No ☐ 97 N/A ☐ 98 Don't know ☐ 99 Refuse to answer

6. During your pregnancy, were you exposed to second-hand smoke in public places?
   (such as bars, arenas, restaurants or bingo halls)
   ☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

BABY'S FATHER SECTION

If the father has signed the consent form, you are allowed to ask these questions; if not, complete the PROTOCOL DEVIATION SECTION.

1. Does the baby's father smoke?
   ☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

   1.1 If *yes*, does the baby's father regularly smoke in the home, that is, every day or almost every day?
   ☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 3 – Visit 3 – Follow-up visit *(between 32 0/7 and 34 6/7 weeks)*

Center ☐☐ ID ☐☐☐ Monogram ☐☐☐

DRINKS AND ALCOHOL CONSUMPTION SECTION *Now some questions about drinks and alcohol.*

1. During the past 3 months, how much did you drink of the following? (Unless otherwise specified, 1 glass = 8 oz, 1 cup = 6 oz)

1.1 Water (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.2 Milk (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.3 Regular coffee (cups)	☐ None	☐☐	cups /	☐ Day ☐ Week ☐ Month
1.4 Decaffeinated coffee (cups)	☐ None	☐☐	cups /	☐ Day ☐ Week ☐ Month
1.5 Regular tea (cups)	☐ None	☐☐	cups /	☐ Day ☐ Week ☐ Month
1.6 Green tea (cups)	☐ None	☐☐	cups /	☐ Day ☐ Week ☐ Month
1.7 Herbal tea (cups)	☐ None	☐☐	cups /	☐ Day ☐ Week ☐ Month
1.8 Apple juice (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.9 Grape juice/cocktail (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.10 Other fruit juice (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.11 Vegetable juice (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month
1.12 Other caffeinated beverages (e.g., colas, iced tea/coffee, chocolate, some sport/energy drinks) (glasses)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month

2. During the past 3 months, have you had a drink of beer, wine, liquor or any other alcoholic beverage?

☐ 1 Yes ☐ 0 No (if no, go to RESIDENCE SECTION) ☐ 99 Refuse to answer

If *yes*, how much did you drink of the following? 2.1 White wine (1 glass = 4 oz)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month ☐ 3 Months
2.2 Red wine (1 glass = 4 oz)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month ☐ 3 Months
2.3 Beer (1 glass = 8 oz)	☐ None	☐☐	glasses /	☐ Day ☐ Week ☐ Month ☐ 3 Months
2.4 Liquor (1 drink = 1 oz)	☐ None	☐☐	drinks /	☐ Day ☐ Week ☐ Month ☐ 3 Months
3. Thinking back over the past 3 months, did you ever consume 5 or more alcoholic drinks on one occasion?	☐ Yes	☐ No		
3.1 If *yes*, how many times?		☐☐	times /	☐ Week ☐ Month ☐ 3 Months

RESIDENCE SECTION - DETAILS

1. Have you moved since visit 1? ☐ 1 Yes ☐ 0 No If no, go to ACTIVITIES SECTION

If yes:

2. What are the first 3 characters of your postal code (if not available, ask about the city name)?

☐☐☐ **or** 2.1 City name _____

3. Which of the following best describes your current home? Is it a …

☐ 0 Detached single family house ☐ 1 Duplex or townhouse ☐ 2 100% residential apartment building
☐ 3 Combined residential and commercial building ☐ 98 Don't know ☐ 99 Refuse to answer

4. Does your home have an attached garage?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

4.1 If *yes*, how many vehicles are usually parked in your garage? ☐☐

5. Is the truck or bus traffic on your street…

☐ 0 Light (occasional trucks/buses passing by) ☐ 1 Medium (many trucks/buses passing by)
☐ 2 Heavy (a continuous flow of trucks/buses) ☐ 98 Don't know

6. Do you ever notice paint chips or dust from paint in your home?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

7. Has your home/apartment had any repairs done in the last 2 years?

☐ 1 Yes ☐ 0 No ☐ 98 Don't know ☐ 99 Refuse to answer

If yes, what type of repairs/renovations occurred in your home? (check all that apply)

7.1 Leaky pipes ☐ 7.2 Holes/Cracks in the Ceiling/Wall ☐ 7.3 Roof repairs ☐
7.4 Leaking basement ☐ 7.5 Don't know ☐ 7.6 Other ☐ 7. 6.1 If other, specify _____

8. Now I'd like to ask some questions about the heating in your home. What is the main type of heating fuel in your home?

☐ 0 Natural Gas ☐ 1 Electric ☐ 2 Fuel oil ☐ 3 Coal ☐ 4 Wood
☐ 5 Other 8.1 If other, specify _____ ☐ 98 Don't know

9. How is your home heated (check all that apply)?

9.1 Radiator (steam or hot water) ☐ 9.2 Forced hot air vents (furnace) ☐ 9.3 Baseboard electric heaters ☐
9.4 Wood stove or fireplace ☐ 9.5 Don't know ☐ 9.6 Other ☐ 9.6.1 If other, specify _____

RESIDENCE SECTION – DETAILS (CONTINUED)

10. Do you have a fireplace/woodstove? ☐ 1 Yes ☐ 0 No

10.1 If *yes*, what do you mostly burn in your fireplace/woodstove?

☐ 0 Natural gas ☐ 1 Propane ☐ 2 Wood or wood pellets ☐ 3 Coal ☐ 4 Corn pellets
☐ 5 Other 10.1.1 If other, specify _____
☐ 98 Don't know ☐ 99 Refuse to answer

11. Where do you get water for general household use (e.g. for showering and cleaning)? (check all that apply)

11.1 Public water system ☐ 11.2 Private well ☐ 11.3 I don't know ☐
11.4 Other source ☐ 11.4.1 If other, specify _____

12. When was your home or apartment built?

☐ 0 Prior to 1960 ☐ 1 1960-1970 ☐ 2 1971-1980 ☐ 3 1981-1990
☐ 4 1991-2000 ☐ 5 2001-2005 ☐ 6 2006 or more recently ☐ 98 Don't know

13. About the cooking appliances, which do you use indoors or outdoors at home (check all that apply)?

13.1 Electric stove ☐ 13.2 Gas stove ☐ 13.3 Wood stove ☐ 13.4 Charcoal BBQ ☐
13.5 Propane/gas BBQ ☐

14. Do you burn candles in your home? ☐ 1 Yes ☐ 0 No

14.1 If *yes*, in the past month (30 days), how many times have you burned candles?

☐☐ times / month ☐ 98 Don't know

15. Do any rooms in your home have wall-to-wall carpets? ☐ 1 Yes ☐ 0 No

If yes, specify (check all that apply):

15.1 Your bedroom ☐ 15.2 Other bedrooms ☐ 15.3 Living room ☐
15.4 Family room ☐ 15.5 Dining room ☐ 15.6 Den/office ☐
15.7 Basement rec room ☐ 15.8 Other ☐ 15.8.1 If other, specify _____

16. Do you have a furnace ☐ 1 Yes ☐ 0 No ☐ 98 Don't know

16.1 If *yes*, how frequently are the filters changed in your furnace?

☐☐ times / ☐ 0 Month ☐ 1 Year ☐ 98 Don't know

ACTIVITIES SECTION

1. In a typical day, how much time (in minutes) do you spend on average? (Fill out all).

1.1 Walking ___ min. 1.2 On a motorcycle / scooter / moped ___ min.
1.3 Biking ___ min. 1.4 In a bus / tram ___ min.
1.5 In a car / taxi ___ min. 1.6 In a train / subway ___ min.

2. In a typical day, how much time (in minutes) do you spend on average directly exposed (within 20 ft) to any vehicle emitting exhaust vapours (e.g. at a bus stop)? ___ min.

3. During this pregnancy, please tell me your 3 most common means of transportation. Start by telling me what mode of transport you take the most, then the 2nd most, and finally, your 3rd most.

CODES
1. Walk
2. Bike
3. Taxi
4. Gas car, truck or SUV
5. Diesel car, truck or SUV
6. Bus /tram
7. Motorcycle / scooter / moped
8. Train/ subway
9. Other

Insert the code

3.1 Most Common ___ 3.1.1 If other, specify _____
3.2 2nd Most ___ 3.2.1 If other, specify _____
3.3 3rd Most ___ 3.3.1 If other, specify _____

4. In a typical week during this pregnancy, how many hours per week do you spend doing household cleaning?

___ 0 Less than 1 hour ___ 1 1 –2 hrs ___ 2 3 –4 hrs ___ 3 5 –7 hrs ___ 4 more than 7 hrs

5. Which rooms in your home are the main ones where cleaning is carried out on a weekly basis (check all that apply)?

5.1 None ___ 5.2 Your bedroom ___ 5.3 Other bedrooms ___ 5.4 Living room ___
5.5 Basement ___ 5.6 Family room ___ 5.7 Dining room ___ 5.8 Kitchen ___
5.9 Bathroom(s) ___ 5.10 Other ___ 5.10.1 If other, specify _____

6. Since the beginning of your pregnancy, would you say that the amount of time you spend housecleaning has…

___ 0 Increased ___ 1 Decreased ___ 2 Stayed the same ___ 98 Don't know ___ 99 Refuse to answer

DIET SECTION

During the last 2 weeks how many times have you eaten [INSERT MEAT] that was cooked [INSERT COOKING METHOD].

	A. Fried	B. Broiled	C. BBQ/Charcoal broiled	D. Cooked so that it is blackened on the outside (by any cooking method)
1. Poultry	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week
2. Hamburger	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week
3. Steak	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week
4. Pork (not including sausage/bacon)	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week
5. Sausage or bacon	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week
6. Fish	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week	☐ Never ☐ times/ ☐ Day ☐ Week

DIET SECTION (CONTINUED)

7. In the past 3 months, how often did you eat the following fish? (Note: each line must be completed)

7.1 Fish sticks	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.2 Tuna packaged in a can or pouch	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.3 Tuna steaks and/or fillets	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.4 Salmon packaged in a can or pouch	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.5 Fresh, frozen, and/or smoked salmon	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.6 Halibut	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.7 Rainbow trout	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.8 Lake trout	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.9 Cod	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.10 Whitefish	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.11 Tilapia	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.12 Shark	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.13 Marlin	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.14 Swordfish	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.15 Orange roughy	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.16 Escolar	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.17 Char	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month

DIET SECTION (CONTINUED)

7.18 Herring	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.19 Mackerel	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.20 Sardines	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.21 Flounder	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.22 Plaice	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.23 Sole	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.24 Pollock	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.25 Haddock	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.26 Other fish 7.26.1 If yes, specify_____	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
7.27 Other fish 7.27.1 If yes, specify_____	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month

8. In the past 3 months, how many servings of fruit or vegetables from your own garden have you consumed?

☐ None ☐☐ Servings / ☐ Day ☐ Week ☐ Month

9. In the past 3 months, how frequently have you eaten coffee-flavoured yogurt or ice cream?

☐ Never ☐☐ Times / ☐ Day ☐ Week ☐ Month

SUNLIGHT EXPOSURE SECTION

1. During the past 4 weeks, how many days did you spend outside for at least 30 minutes between 9 am and 4 pm?

☐☐ Days ☐ None If None, go to question 2.

1.1 When you were outside, how would you describe the coverage of your legs and arms with clothing?

☐ None ☐ Partial ☐ Total

1.2 What was the Sun Protector Factor (SPF) of the main sunscreen that you used?
(the one that you used on the largest exposed area of your body)

☐ 4 ☐ 8 ☐ 15 ☐ 30 ☐ 45

☐ Other 1.2.1 If other, specify_____ ☐ N/A (did not use any sunscreen)

2. During the past 4 weeks, did you use a sunlamp or tanning bed?

☐ Yes ☐ No

ANTHROPOMETRIC MEASUREMENT SECTION

1. Weight measured at this visit ☐☐☐.☐ ☐ 0 Kg ☐ 1 Pounds
If not done, complete the PROTOCOL DEVIATION SECTION

BLOOD PRESSURE AND URINE COLLECTION SECTION

BLOOD PRESSURE MEASUREMENT DURING THIS VISIT (SITTING, LEFT ARM)

1. Have blood pressure measurements been taken? ☐ 1 Yes ☐ 0 No
If no, complete the PROTOCOL DEVIATION SECTION

If yes, record 1.1 1st Measure ☐☐☐ / ☐☐☐ mm/Hg
systolic *diastolic*

One minute apart:

1.2 2nd Measure ☐☐☐ / ☐☐☐ mm/Hg
systolic *diastolic*

BLOOD PRESSURE AND URINE COLLECTION SECTION (CONTINUED)

URINE SAMPLE COLLECTION

Collect a minimum of 65 ml of urine into a 125ml Nalgene container

2. Collection of sample done (CC13) ☐ 1 Yes ☐ 0 No If no or less than expected, complete the PROTOCOL DEVIATION SECTION; if *yes:*

2.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 2.2 Time (24 hr clock) ☐☐ hr ☐☐ min

2.3 Number of hours since last urination (prior to this one) ☐☐ hr ☐☐ min

2.4 Processing and freezing of urine sample done ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If *yes* 2.4.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 2.4.2 Time (24 hr clock) ☐☐ hr ☐☐ min

URINE PROTEIN DIPSTICK TEST Transfer at least 5ml of urine from the 125ml Nalgene container into a 60ml urine container

3. Urine protein test by dipstick done ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

3.1 If *yes*, indicate the result obtained

☐ Zero ☐ 1+ or 30 mg/dL or 0.3 g/L ☐ 3+ or 300 mg/dL or 3.0 g/L

☐ Trace ☐ 2+ or 100 mg/dL or 1.0 g/L ☐ 4+ or 2000 mg/dL or 20 g/L

If protein on dipstick ≥ 1+, check in the patient chart if a 24-hour urine protein test has been ordered in the last seven days:

3.1.1 Was the 24-hour urine test ordered because of a positive dipstick? If not, inform to the patient's doctor the dipstick results and indicate that documentation of a 24-hour urine protein test is highly desirable for the study protocol. If he orders it, mark Yes. If not, specify the reason in the PROTOCOL DEVIATION SECTION.

☐ 1 Yes ☐ 0 No

If *yes*, probe: If the 24-hour urine protein test was ordered today, explain to the patient it should be done within the next 7 days and wait for the results to complete the rest of the section.

3.1.1.1 Indicate the result obtained ☐☐☐☐☐ mg/24 h **or** ☐☐ • ☐ g/L

3.1.1.2 Date test performed *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

BLOOD SAMPLE COLLECTION SECTION

1. Was the woman fasting? ☐ 1 Yes ☐ 0 No

2. Specimen collection done? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

2.1 If *yes* Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐ 2.2 Time (24 hr clock) ☐☐ hr ☐☐ min

2.3 Check tubes collected (3 x10 ml and 2 X 6 ml expected) If less specimens than expected, complete the PROTOCOL DEVIATION SECTION

☐ 10 mL K2-EDTA (CC14) ☐ 10 mL K2-EDTA (CC15) ☐ 6 mL K2-EDTA (CC16) ☐ 6 mL blue tube (CC17) ☐ 10 mL K3-EDTA (CC18)

3. Processing of sample done? ☐ 1 Yes ☐ 0 No 3.1 If yes, time (24 hr clock) ☐☐ hr ☐☐ min

If no, or if delay between collection and centrifugation > 1 hour, complete the PROTOCOL DEVIATION SECTION

4. Specimen frozen? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION. If yes:

4.1 Date of freezing ☐☐ ☐☐☐ ☐☐☐☐ 4.2 Time (24 hr clock) ☐☐ hr ☐☐ min

If delay between collection and freezing > 2 hours, complete the PROTOCOL DEVIATION SECTION

Reminder concerning Blood Specimen Tracking: Information above on specimen collection and storage needs to be included in the specimen log (Excel file)

PROTOCOL DEVIATION SECTION

1. Were there any protocol deviations during this study visit? ☐ 1 Yes ☐ 0 No

If *yes*, specify:

		Study visit	**Specify Reason**
1.1	☐	Study visit done outside the visit window (32 0/7 to 34 6/7)	1.1.1 _____
1.2	☐	A part of the questionnaire was not completed	1.2.1 _____
		Anthropometric measurement	
1.3	☐	Blood pressure measurement not done	1.3.1 _____
1.4	☐	Weight not measured at this visit	1.4.1 _____
		Specimen collection	
1.5	☐	Blood tube not collected	1.5.1 _____
1.6	☐	Blood tube lost	1.6.1 _____
1.7	☐	Blood processing problem	1.7.1 _____
1.8	☐	Blood freezing problem	1.8.1 _____
1.9	☐	Urine protein test by dipstick not done	1.9.1 _____
1.10	☐	24-hour urine protein test not done	1.10.1 _____
1.11	☐	Problem with urine sample collection	1.11.1 _____
1.12	☐	Urine sample freezing problem	1.12.1 _____
		Baby's father information	
1.13	☐	1.13.1 Specify _____	
		Other	
1.14	☐	1.14.1 Specify _____	

INTERVIEWER SECTION

1. Interviewer Initials ☐☐☐ 2. Duration of interview ☐☐☐ min

3. Signature _____ 4. Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

Reminder: Information regarding today's visit needs to be registered in the Follow-up log (Excel file)

UNUSED ALIQUOT LABELS

☐ CHECK HERE IF ALL LABELS HAVE BEEN USED FOR THIS VISIT

If NOT, stick the unused label(s) into the corresponding box(es):

Urine

CC13 (125ml Nalgene)

Label code

48

Label code

49

Label code

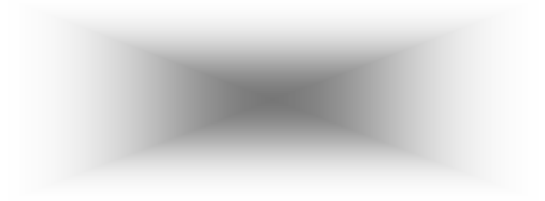
50

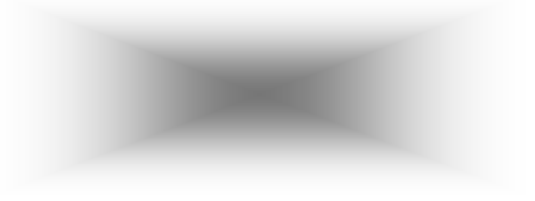
Maternal blood

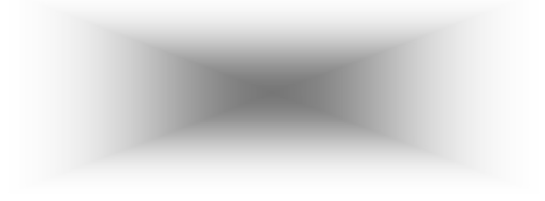
CC14 (10 mL K2-EDTA)

Label code

54







Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

UNUSED ALIQUOT LABELS (CONTINUED)

Maternal blood (continued)

CC15 (10 mL K2-EDTA)		Label code **59**
CC16 (6 mL K2-EDTA)	Label code **60**	Label code **61**
CC17 (6 mL blue tube)	Label code **62**	Label code **63**
	Label code **64**	Label code **65**
	Label code **66**	Label code **67**
CC18 (10 mL K3-EDTA)	Label code **68**	

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 4 – Visit 4 – Delivery

Center | ID | Monogram | Day | Month | Year

VISIT STATUS SECTION

1. Visit done? ☐ 1 Yes ☐ 0 No If no, you must complete this section as well as PREGNANCY STATUS SECTION

If no, specify:

1.1 Woman missed at delivery (if yes, explain the reason in the PROTOCOL DEVIATION SECTION)	☐ 1 Yes	☐ 0 No
1.2 The woman refuses to participate further	☐ 1 Yes	☐ 0 No
1.3 The woman does not want to be contacted by letter or telephone	☐ 1 Yes	☐ 0 No
1.4 The woman refuses access to her medical or hospital records	☐ 1 Yes	☐ 0 No

1.5 Other ☐ 1 Yes ☐ 0 No 1.5.1 If other, specify _____

PREGNANCY STATUS SECTION

1. Multiple pregnancy? ☐ 1 Yes ☐ 0 No 1.1 If yes, number of babies ☐

2.1 Number of babies born alive ☐ 2.2 Number of stillbirths ☐

3. Please document all stillbirths below: **Important:** Baby 1 = first born baby, Baby 2 = second born baby, Baby 3 = third born baby

3.1 *Baby 1* 1. Before labor ☐ If before: 3.1.1 Date ☐☐ ☐☐☐ ☐☐☐☐
2. During labor ☐

3.2 *Baby 2* 1. Before labor ☐ If before: 3.2.1 Date ☐☐ ☐☐☐ ☐☐☐☐
2. During labor ☐

3.3 *Baby 3* 1. Before labor ☐ If before: 3.3.1 Date ☐☐ ☐☐☐ ☐☐☐☐
2. During labor ☐

For all stillbirths: you still need to complete this questionnaire as well as questionnaires *5a-5b* and *7*, and questionnaires *8a-8b* in the case of a *multiple pregnancy*.

URINE SAMPLE COLLECTION SECTION Collect a minimum of 60 ml of urine (CC19)

1. Collection of urine sample done ☐ 1 Yes ☐ 0 No If no or less than expected, complete the PROTOCOL DEVIATION SECTION

If *yes:*

1.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 1.2 Time (24 hr clock) ☐☐ hr ☐☐ min

1.3 Number of hours since last urination (prior to this one) ☐☐ hr ☐☐ min

1.4 Processing and freezing of urine sample done ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If yes 1.4.1 Date of freezing ☐☐ ☐☐☐ ☐☐☐☐ 1.4.2 Time (24 hr clock) ☐☐ hr ☐☐ min

Note: the space for the UNUSED ALIQUOT LABELS for this urine collection is in POST-PARTUM QUESTIONNAIRE (5A)

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 4 – Visit 4 – Delivery

Center ☐☐ ID ☐☐☐ Monogram ☐☐☐

BLOOD SAMPLE COLLECTION SECTION

1. Was the woman fasting? ☐ 1 Yes ☐ 0 No

2. Specimen collection done? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If yes:

2.1 Date ddmmmyyyy ☐☐ ☐☐☐ ☐☐☐☐ 2.2 Time (24 hr clock) ☐☐ hr ☐☐ min

2.3 Check tubes collected (3 x10 ml and 2 X 6 ml expected) If less specimens than expected, complete the PROTOCOL DEVIATION SECTION

☐ 10 mL K2-EDTA (CC20) ☐ 10 mL K2-EDTA (CC21) ☐ 6 mL K2-EDTA (CC22) ☐ 6 mL blue tube (CC23) ☐ 10 mL K3-EDTA (CC24)

3. Processing of sample done? ☐ 1 Yes ☐ 0 No If yes 3.1 Time (24 hr clock) ☐☐ hr ☐☐ min

If no, or if delay between collection and centrifugation > 1 hour, complete the PROTOCOL DEVIATION SECTION

4. Specimen frozen? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If yes 4.1 Date of freezing ddmmmyyyy ☐☐ ☐☐☐ ☐☐☐☐ 4.2 Time ☐☐ hr ☐☐ min

If delay between collection and freezing > 2 hours, complete the PROTOCOL DEVIATION SECTION

Note: the space for the UNUSED ALIQUOT LABELS for this blood collection is in POST-PARTUM QUESTIONNAIRE (5A)

Reminder concerning Blood Specimen Tracking: Information above on specimen collection and storage needs to be included in the specimen log (Excel file)

CORD BLOOD SAMPLE COLLECTION SECTION

If multiple pregnancy, complete the CORD BLOOD SAMPLE COLLECTION SECTION of Questinnaire 8A at the same time

1. **Venous cord blood baby 1:** Collection done? ☐ 1 Yes ☐ 0 No

If no, complete the PROTOCOL DEVIATION SECTION

If yes

1.1 Date dd mmm yyyy ☐☐ ☐☐☐ ☐☐☐☐ 1.2 Time (24 hr clock) ☐☐ hr ☐☐ min

1.3 By: _____

1.4 Check containers collected If less containers than expected, complete the PROTOCOL DEVIATION SECTION

☐ 4.9ml SM (CC25)

☐ Baxter bag (CC26) Note: Extract blood from the Baxter bag to fill out the 9x10ml tubes expected.

☐ 10ml EDTA (CC26.1) ☐ 10ml EDTA (CC26.2) ☐ 10ml EDTA (CC26.3) ☐ 10ml EDTA (CC26.4) ☐ 10ml EDTA (CC26.5) ☐ 10ml EDTA (CC26.6) ☐ 10ml EDTA (CC26.7) ☐ 10ml EDTA (CC26.8) ☐ 10ml EDTA (CC26.9)

2. Processing of cord blood sample done? ☐ 1 Yes ☐ 0 No If yes 2.1 Time ☐☐ hr ☐☐ min

If no, complete the PROTOCOL DEVIATION SECTION

3. Specimen frozen? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If yes 3.1 Date of freezing ☐☐ ☐☐☐ ☐☐☐☐ 3.2 Time ☐☐ hr ☐☐ min

If delay between collection and freezing > 2 hours, complete the PROTOCOL DEVIATION SECTION

Reminder concerning Blood Specimen Tracking: Information above on specimen collection and storage needs to be included in the specimen log (Excel file)

PROTOCOL DEVIATION SECTION

1. Were there any protocol deviations during this study visit? ☐ 1 Yes ☐ 0 No

If yes, specify:

		Study visit	**Specify Reason**
1.1	☐	Women missed at delivery	1.1.1 _____
1.2	☐	A part of the questionnaire was not completed	1.2.1 _____
		Specimen collection	
1.3	☐	Blood tube not collected	1.3.1 _____
1.4	☐	Blood tube lost	1.4.1 _____
1.5	☐	Blood processing problem	1.5.1 _____
1.6	☐	Blood freezing problem	1.6.1 _____
1.7	☐	Problem with cord blood collection	1.7.1 _____
1.8	☐	Cord blood tube lost	1.8.1 _____
1.9	☐	Cord blood processing problem	1.9.1 _____
1.10	☐	Cord blood freezing problem	1.10.1 _____
1.11	☐	Problem with urine sample collection	1.11.1 _____
1.12		Urine sample freezing problem	1.12.1 _____
		Other	
1.13	☐	1.13.1 Specify _____	
1.14	☐	1.14.1 Specify _____	

INTERVIEWER SECTION

1. Interviewer Initials ☐☐☐ 2. Duration of interview ☐☐☐ min

3. Signature _____ 4. Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

Reminder: Information regarding today's visit needs to be registered in the Follow-up log (Excel file)

UNUSED ALIQUOT LABELS

☐ CHECK HERE IF ALL LABELS HAVE BEEN USED FOR THIS VISIT

If NOT, stick the unused label(s) into the corresponding box(es):

Urine: the space for the UNUSED ALIQUOT LABELS for the delivery urine collection is in QUESTIONNAIRE POST-PARTUM (5A).

Maternal blood: the space for the UNUSED ALIQUOT LABELS for the delivery maternal blood collection is in QUESTIONNAIRE POST-PARTUM (5A).

Cord blood

CC25 (S-monovette)

Label code
96

CC26.1 (10mL EDTA)

Label code
97

CC26.2 (10 mL EDTA)

Label code
101

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

UNUSED ALIQUOT LABELS (CONTINUED)

Cord blood (continued)

CC26.3 (10mL EDTA)

Label code

104

CC26.4 (10mL EDTA)

Label code

107

Label code

109

CC26.5 (10 mL EDTA)

Label code

110

Label code

112

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

UNUSED ALIQUOT LABELS (CONTINUED)

Cord blood (continued)

CC26.6 (10mL EDTA)

CC26.7 (10mL EDTA)

CC26.8 (10 mL EDTA)

CC26.9 (10 mL EDTA)

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 5A – Post-partum Questionnaire

Centre | ID | Monogram | Day | Month | Year

VISIT STATUS SECTION

1. Visit done? ☐ 1 Yes ☐ 0 No

If no:

1.1 Woman missed at early postpartum (if yes, explain the reason in the PROTOCOL DEVIATION SECTION) ☐ 1 Yes ☐ 0 No

If *yes* contact the patient to give her the human milk kit for visit 6

1.1.1 Were you able to contact her: ☐ 1 Yes ☐ 0 No

If yes, complete PREPARATION FOR VISIT 6 SECTION (page 3)

If no, specify reason: 1.1.1.1 No answer ☐

1.1.1.2 Telephone disconnected ☐

1.1.1.3 The woman is too busy ☐

1.1.1.4 The woman is ill ☐

1.1.1.5 Other ☐ 1.1.1.5.1 Specify _____

1.2 The woman refuses to participate further ☐ 1 Yes ☐ 0 No

1.3 The woman does not want to be contacted by letter or telephone ☐ 1 Yes ☐ 0 No

1.4 The woman refuses access to her medical or hospital records ☐ 1 Yes ☐ 0 No

1.5 Other ☐ 1 Yes ☐ 0 No 1.5.1 If other, specify _____

CURRENT MEDICATION SECTION

1. Are you taking any medication? Ask for any current medication (ASA, antihypertensive, insulin, hypoglycemic, etc).

☐ 0 **None** If none, go to URINE SAMPLE COLLECTION SECTION

A. Name of the product (When possible, use generic name, except for combination products)	**B. Indication**
1.1A	1.1B
1.2A	1.2B
1.3A	1.3B
1.4A	1.4B
1.5A	1.5B
1.6A	1.6B
1.7A	1.7B
1.8A	1.8B
1.9A	1.9B
1.10A	1.10B

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 4 – Post-partum Questionnaire Visit 4 – Delivery

Centre Center | ID | Monogram Monogram

URINE SAMPLE COLLECTION SECTION Collect a minimum of 60 ml of urine (CC19)

1. Was urine sample collected at delivery *(Questionnaire 4)*? ☐ 1 Yes ☐ 0 No If yes, go to BLOOD SAMPLE COLLECTION SECTION

If urine sample was collected at delivery and there are labels remaining, go to page 5 of this questionnaire.

If urine sample was not collected at delivery, please collect the urine post-partum and complete the information below.

2. Collection of urine sample done at this visit? ☐ 1 Yes ☐ 0 No If no or less than expected, complete the PROTOCOL DEVIATION SECTION

If *yes:*

2.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 2.2 Time (24hr clock) ☐☐ hr ☐☐ min

2.3 Number of hours since last urination (prior to this one) ☐☐ hr ☐☐ min

2.4 Processing and freezing of urine sample done ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If yes 2.4.1 Date of freezing ☐☐ ☐☐☐ ☐☐☐☐ 2.4.2 Time ☐☐ hr ☐☐ min

BLOOD SAMPLE COLLECTION SECTION

1. Was blood sample collected at delivery *(Questionnaire 4)*? ☐ 1 Yes ☐ 0 No If yes, go to MECONIUM SAMPLE COLLECTION SECTION

If blood sample was collected at delivery, but there are labels remaining, go to pages 5, 6, and 7 of this questionnaire.

If blood sample was not collected at delivery, please collect the blood post-partum and complete the information below.

2. Was the woman fasting? ☐ 1 Yes ☐ 0 No

3. Collection of blood sample done at this visit? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If *yes:*

3.1 Date *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐ 3.2 Time (24 hr clock) ☐☐ hr ☐☐ min

3.3 Check tubes collected (3 x 10 ml and 2 X 6 ml expected) If less specimens than expected, complete the PROTOCOL DEVIATION SECTION

☐ 10 mL K2-EDTA (CC20) ☐ 10 mL K2-EDTA (CC21) ☐ 6 mL K2-EDTA (CC22) ☐ 6 mL blue tube (CC23) ☐ 10 mL K3-EDTA (CC24)

4 Processing of sample done? ☐ 1 Yes ☐ 0 No If yes 4.1 Time (24 hr clock) ☐☐ hr ☐☐ min

If no, or if delay between collection and centrifugation > 1 hour, complete the PROTOCOL DEVIATION SECTION

5. Specimen frozen? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If yes 5.1 Date of freezing *ddmmmyyyy* ☐☐ ☐☐☐ ☐☐☐☐ 5.2 Time ☐☐ hr ☐☐ min

If delay between collection and freezing > 2 hours, complete the PROTOCOL DEVIATION SECTION

Reminder concerning Blood Specimen Tracking: Information above on specimen collection and storage needs to be included in the specimen log (Excel file)

MECONIUM SAMPLE COLLECTION SECTION

If multiple pregnancy, complete the MECONIUM SAMPLE COLLECTION SECTION of Questionnaire 8A at the same time

Important: the 2 x 10g of meconium can be collected in one or two occasions

1. Collection of 2 samples of 10 g of meconium done (1) 10g (CC27) ☐ 1 Yes ☐ 0 No
   If 2 x 10g not collected, complete the PROTOCOL DEVIATION SECTION (2) 10g (CC28) ☐ 1 Yes ☐ 0 No

If there was a collection, record the date and time of respective samples:

1.1 (CC27) Date *dd mmm* ☐☐ ☐☐☐ 1.1.1 Time (24 hr clock) ☐☐ hr ☐☐ min

1.2 (CC28) Date *dd mmm* ☐☐ ☐☐☐ 1.2.1 Time (24 hr clock) ☐☐ hr ☐☐ min

2. Freezing of meconium samples done ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If yes 2.1 Freezing date *dd mmm* ☐☐ ☐☐☐ 2.2 Time (24 hr clock) ☐☐ hr ☐☐ min

Reminder concerning Meconium Specimen Tracking: Information above on specimen collection and storage needs to be included in the Specimen Log (Excel file)

PREPARATION FOR VISIT 6 SECTION

1. Is the woman planning to breast feed her baby?
   - ☐ 1 Yes Go to question 2.
   - ☐ 0 No Go to question 3.
2. Does the woman still consent to participate in the human milk component (i.e. at home visit)?
   - ☐ 1 Yes Give Questionnaires 6A and 6B and the jars for milk collection. Then go to page 5.
   - ☐ 0 No Go to question 3.
3. For those women who are not going to participate in the human milk component, proceed to collect a hair sample:

   3.1 Hair collection done ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

   If *yes:*

   3.2 Processing of the hair sample done? (Label bag CC29) ☐ 1 Yes ☐ 0 No
   If no, complete the PROTOCOL DEVIATION SECTION

   If *yes:*

   3.2.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 3.2.2 Time (24 hr clock) ☐☐ hr ☐☐ min

Please complete HAIR SECTION next page immediately after or immediately before collecting the hair sample.

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 5A – Delivery / Postpartum Questionnaire

Center | Centre | Monogram | Monogram

HAIR SECTION

To be completed only if the woman did not consent to participate in the human milk component and hair collection was done.

1. Is your hair currently color treated, permed or straightened with solutions? ☐ 1 Yes ☐ 0 No If no, go to question 2.

If yes, complete the table below. (Note: each line must be completed)

A. What treatment? Is it…		B. Was the treatment purchased over the counter or done at a professional salon?		C. What was the brand of the treatment?		D. Approximately how long ago did you get your hair treated?	
1.1 Colored - Permanent dye	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.2 Colored – Semi-permanent dye	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.3 Highlighted – Full with bleach	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.4 Highlighted – Full without bleach	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.5 Highlighted – Partial with bleach	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.6 Highlighted – Partial without bleach	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.7 Permed	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.8 Straightened with relaxer or other solutions	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months

2. How often in the past year did you swim in a chlorinated or brominated treated pool without wearing a hair cap?

☐☐ times / ☐ Day ☐ Week ☐ Month ☐ Year **or** ☐ Never

PROTOCOL DEVIATION SECTION

1. Were there any protocol deviations during this study visit? ☐ 1 Yes ☐ 0 No

If *yes*, specify

		Specimen collection	**Specify Reason**
1.1	☐	Problem with urine sample collection	1.1.1 _____
1.2	☐	Urine sample freezing problem	1.2.1 _____
1.3	☐	Blood tube not collected	1.3.1 _____
1.4	☐	Blood tube lost	1.4.1 _____
1.5	☐	Blood processing problem	1.5.1 _____
1.6	☐	Blood freezing problem	1.6.1 _____
1.7	☐	Problem with meconium sample collection	1.7.1 _____
1.8	☐	Meconium specimen lost	1.8.1 _____
1.9	☐	Meconium freezing problem	1.9.1 _____
1.10	☐	Problem with hair collection	1.10.1 _____
1.11		Problem with hair sample	1.11.1 _____

Other

1.12 ☐ 1.12.1 Specify _____

1.13 ☐ 1.13.1 Specify _____

INTERVIEWER SECTION

1. Interviewer Initials ☐☐☐ 2. Duration of interview ☐☐☐ min

3. Signature _____ 4. Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

Reminder: Information regarding today's visit needs to be registered in the Follow-up log (Excel file)

UNUSED ALIQUOT LABELS

☐ CHECK HERE IF ALL LABELS HAVE BEEN USED FOR THIS VISIT

If NOT, stick the unused label(s) into the corresponding box(es):

Urine: Labels 71 to 77 are for the **delivery** urine sample collection *(Questionnaire 4)* (**or post-partum**, *if urine was not collected at delivery*)

125ml Nalgene (CC19)

Label code **71**	Label code **72**
Label code **73**	Label code **74**
Label code **75**	Label code **76**
Label code **77**	

Maternal blood: Labels 78 to 95 are for the **delivery** maternal blood sample collection *(Questionnaire 4)* (**or post-partum**, *if maternal blood was not collected at delivery*)

CC20 (10 mL K2-EDTA)

Label code **78**	Label code **79**
Label code **80**	Label code **81**

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

UNUSED ALIQUOT LABELS (CONTINUED)

Maternal blood (continued): Labels 78 to 95 are for the **delivery** maternal blood sample collection *(Questionnaire 4)* (**or post-partum**, *if maternal blood was not collected at delivery*)

CC21 (10 mL K2-EDTA)

Label code **82**	
Label code **84**	

CC22 (6 mL K2-EDTA)

CC23 (6 mL blue tube)

Label code **87**	Label code **88**
Label code **89**	Label code **90**
Label code **91**	Label code **92**

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

UNUSED ALIQUOT LABELS (CONTINUED)

Maternal blood (continued): Labels 78 to 95 are for the **delivery** maternal blood sample collection *(Questionnaire 4)* (**or post-partum**, *if maternal blood was not collected at delivery*)

CC24 (10 mL K3-EDTA)

Label code	Label code
93	**94**

Label code
95

Meconium

CC27 (10g)

Label code
122

CC28 (10g)

Hair: only for the women who are not going to participate in the human milk component

CC29 (bag)

Label code
124

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 5A – Questionnaire for pregnancies ≥ 20 weeks

Centre | ID | Monogram | Day | Month | Year

Please complete this questionnaire based on the information registered in the participant's clinical chart.

GLUCOSE TOLERANCE DURING PREGNANCY SECTION

1. Was a glucose challenge test (GCT) performed during this pregnancy? ☐ 1 Yes ☐ 0 No

If *yes*,

1.1 What was the load of glucose? ☐ 0 50 g ☐ 1 75 g

1.2 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

1.3 Result ☐☐☐ mg/dL **or** ☐ • ☐ mmol/L

2. Was a 75 or 100 g oral glucose tolerance test (OGTT) performed during this pregnancy? ☐ Yes ☐ No

If *yes, specify:*

2.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ ☐ 75g **or** ☐ 100g

Results:

2.2 Fasting ☐☐☐ mg/dL **or** ☐ • ☐ mmol/L

2.3 One hour ☐☐☐ mg/dL **or** ☐ • ☐ mmol/L

2.4 Two hour ☐☐☐ mg/dL **or** ☐ • ☐ mmol/L

2.5 Three hour ☐☐☐ mg/dL **or** ☐ • ☐ mmol/L

CORTICOSTEROIDS DURING PREGNANCY SECTION

1. Did the woman receive corticosteroids during this pregnancy for preventing neonatal respiratory disease?

☐ 1 Yes ☐ 0 No

1.1 If yes, how many doses:

☐ 0 One single dose of 12 mg betamethasone

☐ 1 Single course (2 doses of 12 mg/dose betamethasone in 24 hours)

☐ 2 Weekly course (2 weekly doses of 12 mg/dose betamethasone in 24 hours until 34 weeks or birth)

☐ 3 Other 1.1.1 If other, specify _____

1.2 How long before delivery was the last dose given?

☐ 0 < 24 hrs ☐ 1 ≥ 24 hrs but <7 days ☐ 2 ≥ 7 days

BLOOD PRESSURE DURING PREGNANCY PRIOR TO ADMISSION FOR DELIVERY SECTION (≥ 20 weeks)

1. Highest measure of diastolic blood pressure prior to admission for delivery ☐☐☐ / ☐☐☐ mm/Hg

systolic diastolic

1.1 Date *dd mmm yyyy* ☐☐☐☐☐☐☐☐☐ 1.2 Time ☐☐ hr ☐☐ min

2. Was the woman diagnosed as having gestational hypertension by her doctor prior to admission for delivery (after 20 weeks)?

☐ 1 Yes ☐ 0 No If *yes* 2.1 Date of 1st diagnosis *dd mmm yyyy* ☐☐☐☐☐☐☐☐☐

MIREC Maternal-Infant Research on Environmental Chemicals
Questionnaire 5B, Visit 5, Version 2, March 30 2007

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 5B – Chart Review Questionnaire for pregnancies ≥ 20 weeks

Centre [ ][ ] ID [ ][ ][ ] Monogram [ ][ ][ ]

BLOOD PRESSURE AND URINE TEST AFTER ADMISSION FOR DELIVERY SECTION

1. Highest measure of diastolic blood pressure after admission for delivery [ ][ ][ ] / [ ][ ][ ] mm/Hg
   (systolic / diastolic)

   1.1 Date *dd mmm yyyy* [ ][ ] [ ][ ][ ] [ ][ ][ ][ ] 1.2 Time [ ][ ] hr [ ][ ] min

2. Record the following measure taken **≥ 4 hours later** [ ][ ][ ] / [ ][ ][ ] mm/Hg
   (systolic / diastolic)

   2.1 Date *dd mmm yyyy* [ ][ ] [ ][ ][ ] [ ][ ][ ][ ] 2.2 Time [ ][ ] hr [ ][ ] min

3. Was gestational hypertension diagnosed by the doctor at or after admission [ ] 1 Yes [ ] 0 No

4. Was the woman given antihypertensive agents after admission for delivery [ ] 1 Yes [ ] 0 No

5. Was urine protein by dipstick test done after admission? [ ] 1 Yes [ ] 0 No

   If *yes* 5.1 Indicate the result obtained

   [ ] Zero
   [ ] Trace
   [ ] 1+ or 30 mg/dL or 0.3 g/L
   [ ] 2+ or 100 mg/dL or 1.0 g/L
   [ ] 3+ or 300 mg/dL or 3.0 g/L
   [ ] 4+ or 2000 mg/dL or 20 g/L

6. Was 24-hour urine collection done after admission? [ ] 1 Yes [ ] 0 No

   If *yes* 6.1 Indicate the result obtained [ ][ ][ ][ ][ ] mg/24 h **or** [ ][ ] • [ ] g/L

ANTHROPOMETRIC MEASUREMENT PRIOR TO DELIVERY SECTION

1. Last weight measured prior to delivery [ ][ ][ ] • [ ] [ ] 0 Kg [ ] 1 Pounds

   1.1 Date of measure *dd mmm yyyy* [ ][ ] [ ][ ][ ] [ ][ ][ ][ ]

2. Height recorded in chart [ ][ ][ ] cm **or** [ ] feet [ ][ ] inches [ ] Not recorded

MATERNAL CONDITIONS AFTER ADMISSION SECTION

Did the woman present any of the next listed conditions after admission for delivery?

1. Convulsions – eclampsia [ ] 1 Yes [ ] 0 No

   If *yes* 1.1 Date of first convulsion *dd mmm yyyy* [ ][ ] [ ][ ][ ] [ ][ ][ ][ ]

2. Severe elevation of **diastolic** blood pressure (≥110 mm/Hg) [ ] 1 Yes [ ] 0 No

   If *yes* 2.1 Highest **diastolic** blood pressure value [ ][ ][ ] / [ ][ ][ ] mm/Hg
   (*systolic* / *diastolic*)

   2.2 Date *dd mmm yyyy* [ ][ ] [ ][ ][ ] [ ][ ][ ][ ]

3. Severe elevation of **systolic** blood pressure (≥160 mm/Hg) [ ] 1 Yes [ ] 0 No

   If *yes* 3.1 Highest **systolic** blood pressure value [ ][ ][ ] / [ ][ ][ ] mm/Hg
   (*systolic* / *diastolic*)

   3.2 Date *dd mmm yyyy* [ ][ ] [ ][ ][ ] [ ][ ][ ][ ]

MATERNAL CONDITIONS AFTER ADMISSION SECTION (CONTINUED)

4. Hematocrit < 24.0% ☐ 1 Yes ☐ 0 No ☐ 2 Notdone
If *yes* 4.1 Lowest value ☐☐ • ☐ %
4.2 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

5. Transfusion ☐ 1 Yes ☐ 0 No
If *yes* 5.1 Date of transfusion *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

6. Thrombocytopenia (platelet count < 100 X 10^9/L) ☐ 1 Yes ☐ 0 No ☐ 2 Notdone
If *yes* 6.1 Lowest value ☐☐ x 10^9/L
6.2 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

7. Oliguria (< 500 mL/24 hr) ☐ 1 Yes ☐ 0 No ☐ 2 Notdone
If *yes* 7.1 Lowest value ☐☐☐ mL/24 hr
7.2 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

8. Elevated liver enzyme levels (AST and ALT > 70 U/L) ☐ 1 Yes ☐ 0 No ☐ 2 Notdone
If *yes*, highest value 8.1 AST ☐☐☐ U/L 8.1.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐
8.2 ALT ☐☐☐ U/L 8.2.2 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

9. Pre-delivery hospitalization ☐ 1 Yes ☐ 0 No
If *yes*
9.1 Specify indication(s): 9.1.1 _____
9.1.2 _____
9.1.3 _____

9.2 Number of times admitted to hospital ☐☐ times

9.3 Total number of days of hospitalization prior to admission for delivery ☐☐ days

LABOUR AND DELIVERY SECTION

1. Admission for delivery 1.1 Date *dd mmm yyyy*

1.2 Time hr min

2. Labour onset 0 Spontaneous 1 Induced **or** 2 No labour

Note: induction does not include augmentation of labour with oxytocin

If labour: 2.1 Date of onset *dd mmm yyyy*

2.2 Time of onset (24hr clock) hr min

3. Rupture of membranes

3.1 Date of rupture *dd mmm yyyy*

3.2 Time of rupture (24hr clock) hr min

3.3 Did the woman have spontaneous rupture of membranes prior to the onset of labor 1 Yes 0 No

4. Delivery **baby 1** 4.1 Date *dd mmm yyyy*

4.2 Time (24hr clock) hr min

If more than 1 baby, complete Questionnaire 8A – DELIVERY AND POSTPARTUM QUESTIONNAIRE FOR MULTIPLE PREGNANCY for extra babies

5. Maternal fever during labour (≥ 38.5° C) 1 Yes 0 No

6. Estimated blood loss at delivery (ml) < 500 500 – 999 1 000 - 1 499 ≥ 1 500

7. Method of delivery 0 Spontaneous 1 Instrumental vaginal 2 Cesarean section

If **Cesarean section,** specify indication(s):

7.1 Failure to progress or cephalopelvic disproportion	1 Yes	0 No
7.2 Bleeding	1 Yes	0 No
7.3 Suspected fetal compromise based on abnormal tracing	1 Yes	0 No
7.4 Malpresentation	1 Yes	0 No
7.5 Failed forceps or vacuum	1 Yes	0 No
7.6 Severe hypertensive disorder	1 Yes	0 No
7.7 Previous cesarean section	1 Yes	0 No
7.8 Other	1 Yes	0 No

7. 8.1 If other, specify _____

MATERNAL OUTCOMES AFTER DELIVERY SECTION

1. Maternal fever after delivery ☐ 1 Yes ☐ 0 No (≥ 38.5° C, at least 2 readings more than 24 hours apart, excluding the first 24 hours after delivery)
2. Maternal infection ☐ 1 Yes ☐ 0 No

If *yes*, specify:

2.1 Endometritis	☐ 1 Yes	☐ 0 No
2.2 Episiotomy infection	☐ 1 Yes	☐ 0 No
2.3 Abdominal wound infection	☐ 1 Yes	☐ 0 No
2.4 Urinary tract infection	☐ 1 Yes	☐ 0 No
2.5.Other	☐ 1 Yes	☐ 0 No

2.5.1 If other, specify _____

3. Did the woman present any of the following problems prior to discharge or transfer?

3.1 Antepartum hemorrhage requiring urgent delivery	☐ 1 Yes	☐ 0 No *(without uterine rupture)*
3.2 Disseminated intravascular coagulation	☐ 1 Yes	☐ 0 No
3.3 Pulmonary edema	☐ 1 Yes	☐ 0 No
3.4 ICU admission	☐ 1 Yes	☐ 0 No

If *yes* 3.4.1 Duration ☐☐ day(s) ☐☐ hours 3.4.2 Primary diagnosis _____

3.5 Hysterectomy	☐ 1 Yes	☐ 0 No
3.6 Death	☐ 1 Yes	☐ 0 No

If *yes* 3.6.1 Specify primary contributing cause _____

3. 7 Other maternal complication(s)	☐ 1 Yes	☐ 0 No

If *yes* 3.7.1 Specify _____

4. Was the woman transferred to another hospital? ☐ 1 Yes ☐ 0 No

If *yes:*

4.1 Name of the hospital _____

4.2 Reason of the transfer _____

5. Date and time of transfer/discharge from the delivery hospital (or death)

5.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 5.2. Time (24 hr clock) ☐☐ hr ☐☐ min

NEONATAL INFORMATION baby 1 SECTION

If multiple pregnancy, complete Questionnaire 8B – NEONATAL OUTCOMES FOR MULTIPLE PREGNANCY for extra babies

1. Birthweight ____ g
2. Length at birth ____ . __ cm
3. Cephalic perimeter ____ . __ cm
4. Gender ☐ 0 M ☐ 1 F ☐ 2 Unknown
5. Gestational age at delivery ____ weeks ____ days / 7
6. Placental weight ____ g
7. Did the baby have any congenital anomalies? ☐ 1 Yes ☐ 0 No

   If *yes* 7.1 Specify post-natal diagnosis _____

8. Was the baby born alive ☐ 1 Yes ☐ 0 No

   If *no* 8.1 Was there an autopsy performed? ☐ 1 Yes ☐ 0 No

   8.1.1 If yes, specify diagnosis based on autopsy _____

   8.1.2 If no autopsy, specify clinical diagnosis _____

   If the baby was not born alive, stop data collection here and go to the PROTOCOL DEVIATION SECTION

9. Apgar Score:
   - 9.1 ____ 1 minute ☐ Not done
   - 9.2 ____ 5 minutes ☐ Not done
   - 9.3 ____ 10 minutes ☐ Not done
10. Did the baby have convulsions? ☐ 1 Yes ☐ 0 No
11. Did the baby have respiratory distress? ☐ 1 Yes ☐ 0 No

    If *yes* 11.1 When was respiratory distress first noted:

    ☐ 0 In the first 4 hours after birth ☐ 1 Between 5-24 hours after birth ☐ 2 More than 24 hours after birth

    11.2 Was oxygen therapy required? ☐ 1 Yes ☐ 0 No
12. Did the baby require assisted ventilation? ☐ 1 Yes ☐ 0 No

    If *yes* 12.1 For how long was the baby ventilated:

    ☐ 0 Less than 24 hours ☐ 1 24 hours to 7 days ☐ 2 More than 7 days
13. Was the baby admitted to a neonatal intensive care unit? ☐ 1 Yes ☐ 0 No

    If *yes* 13.1 Length of stay ____ day(s) ____ hours

    13.2 Diagnosis at admission _____
14. Was the baby admitted to an ~~intermediate~~ care ~~unit~~? ☐ 1 Yes ☐ 0 No

    If *yes* 14.1 Length of stay ____ day(s) ____ hours

    14.2 Diagnosis at admission _____

NEONATAL INFORMATION baby 1 SECTION (continued)

15. Did the baby receive systemic antibiotics? ☐ 1 Yes ☐ 0 No

If *yes* 15.1 For what reason were they prescribed _____

16. Was a blood culture done? ☐ 1 Yes ☐ 0 No

If *yes* 16.1 Number of blood cultures done ☐☐

16.2 Culture result: ☐ 1 Positive ☐ 0 Negative 16.2.1 If positive, what species grew _____

17. Did the baby have an intraventricular hemorrhage? ☐ 1 Yes ☐ 0 No

If *yes* 17.1 Grade: **I** ☐ **II** ☐ **III** **IV** ☐ ☐

18. Did the baby have other intracranial hemorrhage? ☐ 1 Yes ☐ 0 No

If *yes* 18.1 Specify _____

19. Did the baby have necrotizing enterocolitis?	☐ 1 Yes	☐ 0 No
20. Did the baby have hypertonia?	☐ 1 Yes	☐ 0 No
21. Did the baby have retinopathy of prematurity?	☐ 1 Yes	☐ 0 No
22. Did the baby have leukomalacia?	☐ 1 Yes	☐ 0 No
23. Did the baby have neutropenia?	☐ 1 Yes	☐ 0 No
24. Did the baby die in the birth hospital?	☐ 1 Yes	☐ 0 No
If *yes* 24.1 Was there an autopsy performed?	☐ 1 Yes	☐ 0 No

24.1.1 If yes, specify diagnosis based on autopsy _____

24.1.2 If no autopsy, specify primary contributing cause _____

25. Other medical problem? ☐ 1 Yes ☐ 0 No

If *yes* 25.1 Specify _____

26. Was the baby transferred to another hospital ☐ 1 Yes ☐ 0 No

If *yes* 26.1 Name of the hospital _____

26.2 Reason of the transfer _____

27. Date and time of transfer/discharge from the birth hospital (or death):

27.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 27.2. Time (24 hr clock) ☐☐ hr ☐☐ min

PROTOCOL DEVIATION SECTION

1. Were there any protocol deviations during this Chart Review? ☐ 1 Yes ☐ 0 No

1.1 If *yes*, specify reason _____

INTERVIEWER SECTION

1. Interviewer Initials ☐☐☐ 2. Duration of review ☐☐☐ min

3. Signature _____ 4. Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

Reminder: Information regarding today's visit needs to be registered in the Follow-up log (Excel file)

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 6 – Visit 6A - Lactational questionnaire *(between 3 and 8 weeks post delivery)*

Center | ID | Monogram | Day | Month | Year

First, let us thank you again for your participation in this landmark study. Your contribution will help us better understand the distribution and the factors that influence nutrients, immunoprotective constituents and environmental chemicals found in human milk.

We would also like to take this opportunity to encourage and support breastfeeding. Breastfeeding is the best method of feeding your infant because it gives optimal nutritional, immunological and emotional benefits for optimal growth and development of your infant.

How to complete the questionnaire

Please complete this questionnaire after you have expressed the requested amount of milk into the jars that were provided. The easiest way to complete the questionnaire is to read and answer the questions in order. The questionnaire will take approximately 15 minutes to complete.

If, for any reason, you are not comfortable answering a question, please write NR near the question. However, we would appreciate you being as accurate as possible in your answers.

We would also like to take this opportunity to tell you that answers to all questions are kept strictly confidential.

If, for any reason, you need some clarification, you may contact the research nurse. The contact information is located on the instructions sheet that you have received.

We would appreciate if your answers are printed clearly.

****

MILK COLLECTION

1. Please indicate the dates of the first and last milk collection for the study:

	DD	MMM (as APR)	YYYY
1.1 Date of first breast milk collection in glass container			
1.2 Date of last breast milk collection in plastic container			

2. For this study, how many occasions and how many days did you need to collect the necessary amount into the glass container?

2.1 ☐☐ Occasions

2.2 ☐☐ Days

3. For this study, how many occasions and how many days did you need to collect the necessary amount into the plastic container?

3.1 ☐☐ Occasions

3.2 ☐☐ Days

4. For this study, when did you mainly give milk? (check all that apply)

4.1 Morning (6:00 am – 11:59 am) ☐

4.2 Afternoon (12:00 pm –5:59 pm) ☐

4.3 Evening (6:00 pm – 11:59 pm) ☐

4.4 Overnight (12:00 am – 5:59 am) ☐

4.5 Equally across all times of the day ☐

MILK COLLECTION (CONTINUED)

5. Which method did you use to express breast milk for the study?

5.1 Hand or manual expression ☐ Yes ☐ No

5.2 Mechanical breast pump ☐ Yes ☐ No If yes 5.2.1 Specify the brand _____

5.3 Electrical breast pump ☐ Yes ☐ No If yes 5.3.1 Specify the brand _____

6. Did you use nipple cream or ointment on the days that you gave milk for the study? ☐ Yes ☐ No

If yes, please specify (check all that apply): 6.1 Breast milk ☐ 6.2 Vitamin E ☐ 6.3 Lanolin ☐

6.4 Other ☐ 6.4.1 If other, specify _____

QUESTIONS ABOUT YOUR BABY

Important: if a multiple pregnancy, this section is for the baby born first.
For the other babies, go to pages 13 & 14 at the end of this questionnaire.
If you didn't breastfeed the baby born first, check this box ☐ and go to page 3 ("QUESTIONS ABOUT YOUR OWN EATING HABITS")

1. What was your baby's weight, length and age at the most recent visit to the doctor's or to the weigh-in clinic?

1.1 What was the visit date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

1.2 Weight of your baby ☐☐ pounds ☐☐ oz **or** ☐☐ • ☐ Kg ☐ I don't know

1.3 Length of your baby ☐☐ ☐ cm / ☐ inches ☐ I don't know

1.4 How old was your baby ☐☐ ☐ days / ☐ weeks ☐ I don't know

2. In the last 7 days, did you give your baby liquids or solid foods (including water, even if a little bit) in addition to breast milk?

☐ Yes ☐ No

If yes

2.1 Specify (check all that apply):

2.1.1 Water (even if only little bit) ☐ 2.1.2 Formula ☐ 2.1.3 Juice ☐ 2.1.4 Solid foods ☐

2.2 How many feedings have included liquids or solid foods?

☐ 1 or 2 ☐ 3 or more ☐ I don't know

3. Thinking back since the birth of your child up until 7 days ago, did you give your baby liquids or solid foods (including water, even if a little bit) in addition to breast milk?

☐ Yes ☐ No

If yes, specify (check all that apply):

3.1 Water (even if only little bit) ☐ 3.2 Formula ☐ 3.3 Juice ☐ 3.4 Solid foods ☐

4. How many more months/weeks are you planning to give breast milk? ☐☐ Months ☐ Weeks ☐ I don't know

5. Since birth, has your baby had any infections which were diagnosed by a doctor? ☐ Yes ☐ No

If yes, check all that apply:

5.1 Ear infection ☐ 5.2 Respiratory infection ☐ 5.3 Digestive tract infection ☐

5.4 Urinary tract infection ☐ 5.5 Other ☐ 5.5.1 If other, specify _____ 5.6 I don't know ☐

QUESTIONS ABOUT YOUR OWN EATING HABITS

The next questions are about the foods you usually eat or drink. Think about all the foods you eat, both meals and snacks, at home and away from home.

1. Since you began feeding your child breast milk, which of these animal products did you eat? (check all that apply)

1.1 Eggs ☐ 1.2 Dairy products ☐ 1.3 Poultry ☐ 1.4 Fish ☐ 1.5 Red meats ☐

1.6 I ate no animal products ☐

2. Since you began feeding your child breast milk, how often did you eat the following fish?

2.1 Fish sticks	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.2 Tuna packaged in a can or pouch	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.3 Tuna steaks and/or fillets	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.4 Salmon packaged in a can or pouch	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.5 Fresh, frozen, and/or smoked salmon	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.6 Halibut	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.7 Rainbow trout	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.8 Lake trout	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.9 Cod	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.10 Whitefish	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.11 Tilapia	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.12 Shark	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.13 Marlin	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.14 Swordfish	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.15 Orange roughy	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month

QUESTIONS ABOUT YOUR OWN EATING HABITS (CONTINUED)

2.16 Escolar	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.17 Char	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.18 Herring	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.19 Mackerel	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.20 Sardines	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.21 Flounder	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.22 Plaice	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.23 Sole	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.24 Pollock	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.25 Haddock	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.26 Other fish 2.26.1 If yes, specify_____	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month
2.27 Other fish 2.27.1 If yes, specify_____	☐ Never	☐☐ times /	☐ Day ☐ Week ☐ Month

3. Each time you eat fish, please approximate your serving size for each of the following items:

3.1 Fish packaged in a can or pouch

☐☐☐ grams **or** ☐☐•☐ oz (1 can of fish = 120g or 4.2 oz) ☐ I do not eat this item

3.2 Fish (other than in a can or pouch)

☐☐☐ grams **or** ☐☐•☐ oz (1 deck of cards = 100g or 3.5 oz) ☐ I do not eat this item

QUESTIONS ABOUT YOUR OWN EATING HABITS (CONTINUED)

4. Since you began feeding your child breast milk, what type of canned or pouched tuna have you usually eaten?
(please choose one)

☐ Light (flaked or chunk) ☐ White (solid) ☐ Both
☐ I did not eat canned or pouched tuna ☐ I don't know

5. Of the red meats you ate since you began feeding your child breast milk, did you eat the visible fat of the meat?
(please choose one)

☐ Always ☐ Sometimes ☐ Never ☐ I did not eat red meat

6. Of the poultry you ate since you began feeding your child breast milk, did you eat the skin of the poultry?
(please choose one)

☐ Always ☐ Sometimes ☐ Never ☐ I did not eat poultry

7. What type of oil do you usually use in cooking, baking or frying? (check all that apply)

7.1 Canola oil ☐ 7.2 Coconut oil ☐ 7.3 Corn oil ☐ 7.4 Olive oil ☐
7.5 Peanut oil ☐ 7.6 Sesame oil ☐ 7.7 Soybean oil ☐ 7.8 Sunflower oil ☐
7.9 Other ☐ 7.9.1 If other, specify: _____

QUESTIONS ABOUT YOUR OWN EATING HABITS (CONTINUED)

8. Since you began feeding your child breast milk, how much did you drink of the following?
(unless otherwise specified, 1 glass=8 oz, 1 cup=6 oz)

8.1 Water (glasses)	☐ None	☐☐ glasses /	☐ Day ☐ Week ☐ Month
8.2 Regular coffee (cups)	☐ None	☐☐ cups /	☐ Day ☐ Week ☐ Month
8.3 Decaffeinated coffee (cups)	☐ None	☐☐ cups /	☐ Day ☐ Week ☐ Month
8.4 Regular tea (cups)	☐ None	☐☐ cups /	☐ Day ☐ Week ☐ Month
8.5 Green tea (cups)	☐ None	☐☐ cups /	☐ Day ☐ Week ☐ Month
8.6 Herbal tea (cups)	☐ None	☐☐ cups /	☐ Day ☐ Week ☐ Month
8.7 Apple juice (glasses)	☐ None	☐☐ glasses /	☐ Day ☐ Week ☐ Month
8.8 Grape juice/cocktail (glasses)	☐ None	☐☐ glasses /	☐ Day ☐ Week ☐ Month
8.9 Other fruit juice (glasses)	☐ None	☐☐ glasses /	☐ Day ☐ Week ☐ Month
8.10 Vegetable juice (glasses)	☐ None	☐☐ glasses /	☐ Day ☐ Week ☐ Month
8.11 Other caffeinated beverages (e.g., colas, iced tea/coffee, chocolate, some sport/energy drinks) (glasses)	☐ None	☐☐ glasses /	☐ Day ☐ Week ☐ Month

9. Since you began feeding your child breast milk, how much did you drink of the following alcoholic beverages?

9.1 White wine (1 glass = 4 oz)	☐ None	☐☐ glasses /	☐ Day ☐ Week ☐ Month
9.2 Red wine (1 glass = 4 oz)	☐ None	☐☐ glasses /	☐ Day ☐ Week ☐ Month
9.3 Beer (1 glass = 8 oz)	☐ None	☐☐ glasses /	☐ Day ☐ Week ☐ Month
9.4 Liquor (1 drink = 1 oz)	☐ None	☐☐ drinks /	☐ Day ☐ Week ☐ Month

10. What is the principal source of the water you drink at home?

☐ Municipal (or city/town) ☐ Private well ☐ Surface source such as lake or river ☐ Bottled water
☐ I don't know ☐ Other source 10.1 If other, specify _____

QUESTIONS ABOUT YOUR NUTRIENT SUPPLEMENTS

Since you began feeding your child breast milk, have you supplemented your diet with one or more of the vitamins, minerals and/or oils listed below? Please complete the table by indicating the drug identification number (or DIN, NPN, NPN-HM), how much of each supplement was taken at each occasion and how often each supplement was taken. Please use the example to complete the rest.

Important: some products do not have a drug identification number. In this case, check "No ID on bottle". Do not write down the lot number or the date of expiration.

EXAMPLE:

5 **A. Vitamin D** **(Specify brand:** Jamieson **)** ☐ **Not used**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): 1234567 ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) 1 Tablet(s)
☐ Other If other, specify: _____

D. Frequency

0 1 times / ☒ day ☐ week ☐ month

---

1 **A. Multivitamin** **(Specify brand:** _____ **)** ☐ **Not used**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

---

2 **A. Calcium** **(Specify brand:** _____ **)** ☐ **Not used**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

---

3 **A. Iron** **(Specify brand:** _____ **)** ☐ **Not used**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

QUESTIONS ABOUT YOUR NUTRIENT SUPPLEMENTS (CONTINUED)

4 **A. Folate or folic acid** **(Specify brand:_____)** ☐ **Not used**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

5 **A. Vitamin D** **(Specify brand:_____)** ☐ **Not used**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

6 **A. Vitamin E** **(Specify brand:_____)** ☐ **Not used**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

7 **A. Fish oil** (cod liver oil, etc.) **(Specify brand: _____)** ☐ **Not used**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

8 **A. Flaxseed** **(Specify brand:_____)** ☐ **Not used**

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)

☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency

☐☐ times / ☐ day ☐ week ☐ month

QUESTIONS ABOUT YOUR NUTRIENT SUPPLEMENTS (CONTINUED)

9 **A. Other supplement: please specify name and brand** _____

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)
☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency
☐☐ times / ☐ day ☐ week ☐ month

10 **A. Other supplement: please specify name and brand** _____

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)
☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency
☐☐ times / ☐ day ☐ week ☐ month

11 **A. Other supplement: please specify name and brand** _____

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)
☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency
☐☐ times / ☐ day ☐ week ☐ month

12 **A. Other supplement: please specify name and brand** _____

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)
☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency
☐☐ times / ☐ day ☐ week ☐ month

13 **A. Other supplement: please specify name and brand** _____

B. Drug identification number on bottle (DIN / NPN / NPN-HM): ☐ **No ID on bottle**

C. Amount taken each time (# of pills, tabs, caps, teaspoon, etc.)
☐ ml ☐ Drop(s) ☐ Teaspoon(s)
☐ Tablespoon(s) ☐ Capsule(s) ☐ Tablet(s)
☐ Other If other, specify: _____

D. Frequency
☐☐ times / ☐ day ☐ week ☐ month

QUESTIONS ABOUT YOUR HOME ENVIRONMENT

1. Have you moved since you gave birth? ☐ Yes (Go to next question) ☐ No (Go to question 6)

2. Describe the house/apartment in which you currently live:

2.1 Age of house or apartment building (years)

☐ <1 ☐ 1-10 ☐ 11-30 ☐ 31+ ☐ I don't know

2.2 Number of bedrooms in your house or apartment

☐ 1 ☐ 2 ☐ 3 ☐ 4+

2.3 Do you live in a house or an apartment? ☐ House ☐ Apartment

2.3.1 If you live <u>in a house:</u> does it have a basement? ☐ Yes ☐ No

2.3.2 If you live <u>in an apartment:</u> what floor do you live on? ☐☐

3. Is the truck or bus traffic on the street…

☐ Light (occasional trucks/buses passing by) ☐ Medium (many trucks/buses passing by)

☐ Heavy (a continuous flow of trucks/buses) ☐ I don't know

4. Where do you get water <u>for general household use</u> (e.g. for showering and cleaning)? (check all that apply)

4.1 Public water system ☐ 4.2 Private well ☐ 4.3 I don't know ☐

4.4 Other source ☐ 4.4.1 If other, specify _____

5. What type of flooring do you have in your living room and bedroom? (check all that apply)

5.1 Vinyl sheet flooring ☐ 5.2 Hardwood floors ☐ 5.3 Wood laminate flooring ☐

5.4 Wall-to-wall carpet ☐ 5.5 I don't know ☐

5.6 Other If other 5.6.1 Specify _____

<u>If you have wall-to-wall carpet</u>:

5.4.1 Approximately how old is the carpet?

☐ Less than 1 year old ☐ 1 to 10 years old ☐ Over 10 years old ☐ I don't know

6. Currently, how many people including yourself live in your home?

☐☐ People under 18 years of age (including your baby) ☐☐ People 18 and over

QUESTIONS ABOUT YOUR SUNLIGHT EXPOSURE

1. During the past 4 weeks, how many days did you spend outside for at least 30 minutes between 9 am and 4 pm?
   ☐☐ Days ☐ None If None, go to question 2.
   1.1 When you were outside, how would you describe the coverage of your legs and arms with clothing?
   ☐ None ☐ Partial ☐ Total
   1.2 What was the Sun Protector Factor (SPF) of the main sunscreen that you used?
   (the one that you used on the largest exposed area of your body)
   ☐ 4 ☐ 8 ☐ 15 ☐ 30 ☐ 45
   ☐ Other 1.2.1 If other, specify _____ ☐ N/A (I did not use any sunscreen)
2. During the past 4 weeks, did you use a sunlamp or tanning bed?
   ☐ Yes ☐ No

A LITTLE MORE PERSONAL QUESTIONS ABOUT YOU

1. What is your weight today? ☐☐☐.☐ ☐ Kg ☐ Pounds ☐ I don't know
2. Approximately how much weight did you gain during this pregnancy? ☐☐☐.☐ ☐ Kg ☐ Pounds ☐ I don't know
3. Was your mother born in Canada? ☐ Yes ☐ No ☐ I don't know
4. Did your mother breastfeed you? ☐ Yes ☐ No ☐ I don't know

Since you began feeding your child breast milk, how often do you:

5. Wear nail polish	☐ Never	☐ Rarely	☐ Sometimes	☐ Often
6. Wear make-up	☐ Never	☐ Rarely	☐ Sometimes	☐ Often
7. Color your hair	☐ Never	☐ Rarely	☐ Sometimes	☐ Often
8. Apply hair relaxers	☐ Never	☐ Rarely	☐ Sometimes	☐ Often
9. Perm your hair	☐ Never	☐ Rarely	☐ Sometimes	☐ Often
10. Use hairstyling products	☐ Never	☐ Rarely	☐ Sometimes	☐ Often
11. Use fragrances, perfumes or colognes	☐ Never	☐ Rarely	☐ Sometimes	☐ Often

12. Currently, how many mercury-silver (also known as amalgam) dental fillings do you have?
    ☐ 0 ☐ 1-4 ☐ 5-9 ☐ 10-14 ☐ 15+ ☐ I don't know
13. Since your study visit during the 3rd trimester, have you had any mercury-silver (also known as amalgam) dental fillings replaced? ☐ Yes ☐ No

A LITTLE MORE PERSONAL QUESTIONS ABOUT YOU (CONTINUED)

14. Are you currently taking any prescription drugs?

☐ Yes ☐ No

If yes, please list:

14.1 _____

14.2 _____

14.3 _____

14.4 _____

14.5 _____

Finally, we would like to know about cigarette smoking. By cigarettes, we mean both ready-made cigarettes and ones you roll yourself.

15. At the present time, do you smoke cigarettes daily, occasionally or not at all?

☐ Daily ☐ Occasionally ☐ Not at all ☐ I prefer not to answer

16. On average, how many cigarettes did you smoke since you gave birth?
(for example: once a day, three times a week, twice a month)

☐☐☐ Cigarettes per ☐ Day ☐ Week ☐ Month ☐ I prefer not to answer

17. Since you began feeding your child breast milk, how often have you been exposed to second hand smoke inside your home?
(For example: once a day, three times a week, twice a month)

☐☐ Times per ☐ Day ☐ Week ☐ Month ☐ Never ☐ I don't know ☐ I prefer not to answer

THANK YOU ! We appreciate your time and effort, and we thank you for your cooperation. We would like again to take the opportunity to tell you that breast milk is naturally the superior food for your infant. We strongly support your decision to breastfeed your baby. After you have filled out the questionnaire, please contact the nurse who distributed the kit to you.

Reminder: in the case of a multiple pregnancy, go to page 13.

FOR MULTIPLE PREGNANCIES ONLY

QUESTIONS ABOUT YOUR BABY – FOR THE BABY BORN SECOND

Important: this page is for **the baby born second only**. In the case of triplets, go to next page for the baby born third.

If you didn't breastfeed the baby born second, check this box and stop here. ☐

1. What was your baby's weight, length and age at the most recent visit to the doctor's or to the weigh-in clinic?

1.1 What was the visit date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

1.2 Weight of your baby ☐☐ pounds ☐☐ oz **or** ☐☐•☐ Kg ☐ I don't know

1.3 Length of your baby ☐☐ ☐ cm ☐ inches ☐ I don't know

1.4 How old was your baby ☐☐ ☐ days ☐ weeks ☐ I don't know

2. In the last 7 days, did you give your baby liquids or solid foods (including water, even if a little bit) in addition to breast milk?

☐ Yes ☐ No

If yes

2.1 Specify (check all that apply):

2.1.1 Water (even if only little bit) ☐ 2.1.2 Formula ☐ 2.1.3 Juice ☐ 2.1.4 Solid foods ☐

2.2 How many feedings have included liquids or solid foods?

☐ 1 or 2 ☐ 3 or more ☐ I don't know

3. Thinking back since the birth of your child up until 7 days ago, did you give your baby liquids or solid foods (including water, even if a little bit) in addition to breast milk?

☐ Yes ☐ No

If yes, specify (check all that apply):

3.1 Water (even if only little bit) ☐ 3.2 Formula ☐ 3.3 Juice ☐ 3.4 Solid foods ☐

4. How many more months/weeks are you planning to give breast milk? ☐☐ Months ☐ Weeks ☐ I don't know

5. Since birth, has your baby had any infections which were diagnosed by a doctor? ☐ Yes ☐ No

If yes, check all that apply:

5.1 Ear infection ☐ 5.2 Respiratory infection ☐ 5.3 Digestive tract infection ☐

5.4 Urinary tract infection ☐ 5.5 Other ☐ 5.5.1 If other, specify_____ 5.6 I don't know ☐

In the case of twins, stop data collection here. If triplets, go to page 14.

FOR MULTIPLE PREGNANCIES ONLY

QUESTIONS ABOUT YOUR BABY – FOR THE BABY BORN THIRD

Important: this page is for **the baby born third only**.

If you didn't breastfeed the baby born third, check this box and stop here. ☐

1. What was your baby's weight, length and age at the most recent visit to the doctor's or to the weigh-in clinic?

1.1 What was the visit date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

1.2 Weight of your baby ☐☐ pounds ☐☐ oz **or** ☐☐ • ☐ Kg ☐ I don't know

1.3 Length of your baby ☐☐ ☐ cm ☐ inches ☐ I don't know

1.4 How old was your baby ☐☐ ☐ days ☐ weeks ☐ I don't know

2. In the last 7 days, did you give your baby liquids or solid foods (including water, even if a little bit) in addition to breast milk?

☐ Yes ☐ No

If yes

2.1 Specify (check all that apply):

2.1.1 Water (even if only little bit) ☐ 2.1.2 Formula ☐ 2.1.3 Juice ☐ 2.1.4 Solid foods ☐

2.2 How many feedings have included liquids or solid foods?

☐ 1 or 2 ☐ 3 or more ☐ I don't know

3. Thinking back since the birth of your child up until 7 days ago, did you give your baby liquids or solid foods (including water, even if a little bit) in addition to breast milk?

☐ Yes ☐ No

If yes, specify (check all that apply):

3.1 Water (even if only little bit) ☐ 3.2 Formula ☐ 3.3 Juice ☐ 3.4 Solid foods ☐

4. How many more months/weeks are you planning to give breast milk? ☐☐ Months ☐ Weeks ☐ I don't know

5. Since birth, has your baby had any infections which were diagnosed by a doctor? ☐ Yes ☐ No

If yes, check all that apply:

5.1 Ear infection ☐ 5.2 Respiratory infection ☐ 5.3 Digestive tract infection ☐

5.4 Urinary tract infection ☐ 5.5 Other ☐ 5.5.1 If other, specify_____ 5.6 I don't know ☐

MIREC Maternal-Infant Research on Environmental Chemicals

Questionnaire 6B – Milk collection sheet

Center | ID | Monogram

How to complete the milk collection sheet

Each time you give milk for the study, please complete one line on the sheet. For each collection, indicate the date, the time of the day, the method (hand or pump), which breast was used, if it was the fore or hind milk from the given breast and whether the milk was collected in the glass or plastic jar. Please use as many lines as needed.

Important instructions

Please use the following date formats: January=JAN February=FEB March = MAR April=APR May=MAY June=JUN July=JUL August=AUG September=SEP October=OCT November=NOV December=DEC

Definition of **fore** (first) milk: in the table below, fore milk refers to the milk expressed from a given breast before the baby drank from that breast.

Definition of **hind** (last) milk: in the table below, hind milk refers to the milk expressed from a given breast after the baby drank from that breast.

Example

Collection 1 - You gave milk for the study by hand expressing on Sunday, February 3rd, 2008 at 10:30am. The baby took all the milk from the right breast, and some from the left one. The milk collected for the study was from the left breast, after the baby had fed on it. This is what we call the hind (or "last") milk.

Collection 2 - Later that day (7:00pm), you expressed milk using a pump. You started by feeding your baby with milk pumped from the left breast. Your baby drank all he needed and fell asleep. Then you expressed the rest of the milk from the left breast. This is considered the "hind" milk. Next, you pumped milk from your right breast for the study. Since your baby had not drunk from the right breast, this was the fore (or "first") milk from it. This means you collected both the fore and hind milk for the study during this collection. You used the glass collection jar for both collections, since you were just starting collecting for the study.

Based on this example, the sheet should be completed as follows:

	Date of milk collection			**Time of day?**				**Hand or pump?**		**Which breast?**			**Fore (first) or hind (last) milk?**			**Glass or plastic jar?**	
				morning *6:00am-11:59 am*	afternoon *12:00pm-5:59 pm*	evening *6:00pm-11:59 pm*	overnight *12:00am-5:59 am*										
	DD	MMM	YYYY					Hand	Pump	Left	Right	Both	Fore	Hind	Both	Glass	Plastic
1.	03	FEB	2008	x				x		x				x		x	
2.	03	FEB	2008			x			x			x			x	x	

****

Please complete the sheet by following the example given above. Thank you.

MIREC Maternal-Infant Research on Environmental Chemicals

Questionnaire 6B – Milk collection sheet

Center ☐☐ ID ☐☐☐ Monogram ☐☐☐

	Date of milk collection			Time of day?				Hand or pump?		Which breast?			Fore (first) or hind (last) milk?			Glass or plastic jar?	
				morning *6:00am-11:59 am*	afternoon *12:00pm-5:59 pm*	evening *6:00pm-11:59 pm*	overnight *12:00am-5:59 am*										
	DD	MMM	YYYY					Hand	Pump	Left	Right	Both	Fore	Hind	Both	Glass	Plastic
1.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
2.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
3.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
4.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
5.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
6.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
7.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
8.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
9.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
10.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
11.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
12.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
13.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

MIREC Maternal-Infant Research on Environmental Chemicals

Questionnaire 6B – Milk collection sheet

Center ☐☐ ID ☐☐☐ Monogram ☐☐☐

	Date of milk collection			Time of day?				Hand or pump?		Which breast?			Fore (first) or hind (last) milk?			Glass or plastic jar?	
				morning	afternoon	evening	overnight										
	DD	MMM	YYYY	*6:00am-11:59 am*	*12:00pm-5:59 pm*	*6:00pm-11:59 pm*	*12:00am-5:59 am*	Hand	Pump	Left	Right	Both	Fore	Hind	Both	Glass	Plastic
14.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
15.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
16.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
17.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
18.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
19.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
20.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
21.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
22.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
23.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
24.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
25.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐
26.	☐☐	☐☐☐	☐☐☐☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐	☐

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 6 – Visit 6C - Lactational questionnaire *(between 3 and 8 weeks post delivery)*

HOME VISIT

Center | ID | Monogram | Day | Month | Year

INTRODUCTION TO QUESTIONNAIRE 6C SECTION

1. Questionnaire 6A was obtained from the participant ☐ 1 Yes ☐ 0 No
   If no, complete the PROTOCOL DEVIATION SECTION
2. I verified the questionnaire 6A with the participant to ensure there is no missing data ☐ 1 Yes ☐ 0 No
   If no, complete the PROTOCOL DEVIATION SECTION
3. A part of the questionnaire 6A is not completed ☐ 1 Yes ☐ 0 No
   If yes, complete the PROTOCOL DEVIATION SECTION
4. Questionnaire 6B (milk collection sheet) was obtained from the participant ☐ 1 Yes ☐ 0 No
   If no, complete the PROTOCOL DEVIATION SECTION

ANTHROPOMETRIC MEASUREMENTS SECTION

1. Last known baby's weight (Clinic) ☐☐.☐ ☐ Pounds ☐ Kg ☐ 98 Don't know
2. Last known baby's length (Clinic) ☐☐ ☐ inches ☐ cm ☐ 98 Don't know
3. Date of last measurements *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ ☐ 98 Don't know

MATERNAL MILK SAMPLE SECTION

1. Maternal milk sample returned? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION
2. Expected quantity collected?
   - 2.1 Glass jar (CC30) (110 ml expected): ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION
   - 2.2 Plastic jar (CC31) (90 ml expected): ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION
3. Milk sample frozen when collected at home visit? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

HAIR SAMPLE COLLECTION SECTION

1. Hair collection done? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If *yes*:

1.1 Processing of the sample done? (Label bag CC29) ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If *yes:*

1.1.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 1.1.2 Time (24 hr clock) ☐☐ hr ☐☐ min

Please complete HAIR SECTION next page immediately after or immediately before collecting the hair sample.

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 6 – Visit 6C - Lactational questionnaire *(between 3 and 8 weeks post delivery)* HOME VISIT

Centre ☐☐ ID ☐☐☐ Monogram ☐☐☐

HAIR SECTION

THIS PAGE NEEDS TO BE COMPLETED ONLY IF THE HAIR COLLECTION WAS DONE.

1. Is your hair currently color treated, permed or straightened with solutions? ☐ 1 Yes ☐ 0 No If no, go to question 2.

If yes, complete the table below. (Note: each line must be completed)

A. What treatment? Is it…		B. Was the treatment purchased over the counter or done at a professional salon?		C. What was the brand of the treatment?		D. Approximately how long ago did you get your hair treated?	
1.1 Colored - Permanent dye	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.2 Colored – Semi-permanent dye	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.3 Highlighted – Full with bleach	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.4 Highlighted – Full without bleach	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.5 Highlighted – Partial with bleach	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.6 Highlighted – Partial without bleach	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.7 Permed	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months
1.8 Straightened with relaxer or other solutions	☐ Yes ☐ No	If yes:	☐ Over the counter ☐ Salon ☐ Don't know	_____	☐ Don't know	☐☐	☐ Days ☐ Weeks ☐ Months

2. How often in the past year did you swim in a chlorinated or brominated treated pool without wearing a hair cap?

☐☐ times / ☐ Day ☐ Week ☐ Month ☐ Year **or** ☐ Never

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 6 – Visit 6C - Lactational questionnaire *(between 3 and 8 weeks post delivery)*

HOME VISIT

Center ☐☐ ID ☐☐☐ Monogram ☐☐☐

FOOD FREQUENCY QUESTIONNAIRE SECTION

In the next part of the interview, we are interested in knowing whether you ate certain foods over the last month. If you did eat them, we would like to know how often you ate them. We are interested only in whether you have eaten them in the last month. So, if you have not eaten those foods at least once within the past month (that is since..... give the date of 1 month ago), they are not important to this part of the interview.

"Please think about the last 4 weeks. Were they work weeks or holidays times? Did they involve a special occasion such as a wedding or party?"

"Now I am going to read the list of foods"

"In the past month did you consume_____?"

IF NO, MOVE TO NEXT FOOD.

IF YES, THEN GO TO THE NEXT COLUMN "B. FREQUENCY" and THEN, ASK SERVING SIZE IN COLUMN "C"

A. FOOD		B. FREQUENCY	C. SERVING SIZE	
In the past month, did you consume:		How often did you consume it per day, week or month?	Using the average serving size as a guide, would your usual serving size be:	**Average Serving**
Vegetables				
1. Broccoli	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
2. Spinach - raw	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
3. Spinach - cooked	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
4. Sweet peppers *(any colours, fresh or processed)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup or ½ pepper
5. Tomatoes *(fresh or canned, incl. tomato based sauce)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 tomato or 125 ml or ½ cup
6. Tomato or vegetable juice	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
7. Potatoes with the skin *(skin eaten)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 medium potato
Fruits				
8. Citrus fruits – excl. juices	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 orange or ½ grapefruit

FOOD FREQUENCY QUESTIONNAIRE SECTION (continued)

A. FOOD		B. FREQUENCY	C. SERVING SIZE	
In the past month, did you consume:		How often did you consume it per day, week or month?	Using the average serving size as a guide, would your usual serving size be:	**Average Serving**
Fruits **(continued)**				
9. Orange juice with calcium	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
10. Other fruit juices and drinks	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
Meat, Poultry, Fish and Alternatives				
11. Red meat *(beef, hamburger, pork, lamb)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz or 1 patty
12. Poultry *(chicken, turkey)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz
13. Wild game – animals	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz
14. Wild game – birds	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz
15. Liver	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz
16. Luncheon meats *(cold cuts)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	3 slices
17. Sausages or wieners *(with or without a bun)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 link
18. Bacon	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	3 slices
19. Eggs and egg dishes	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	2 eggs
20. Fish – excl. shellfish *(fish sticks, tuna, salmon, trout, etc.)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	75 g or 2.5 oz
21. Clams and oysters	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	6 clams or oysters

FOOD FREQUENCY QUESTIONNAIRE SECTION (continued)

A. FOOD		B. FREQUENCY	C. SERVING SIZE	
In the past month, did you consume:		How often did you consume it per day, week or month?	Using the average serving size as a guide, would your usual serving size be:	**Average Serving**
Meat, Poultry, Fish and Alternatives **(continued)**				
22. Other shellfish *(shrimp, lobster, crab, etc.)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	10 medium shrimp or 75 g or 2.5 oz
23. Beans and dry peas *(baked beans, pea soup, chilli, hummus, lentils, etc.)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
Milk Products				
24. Milk (*whole, 2%, 1%, skim, chocolate, Lactaid)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
25. Soy and rice beverages fortified with calcium and vitamin D	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
26. Hard cheese *(parmesan, romano)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	30 ml or 2 tablespoons shredded
27. Cheese – Regular, low fat or partly skimmed *(e.g. mozzarella and cheddar)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	30 g or 1 oz
28. Processed cheese *(cheese slices or spread)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 slice or 2 tablespoons
29. Cottage cheese	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
30. Cream cheese	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	30 ml or 2 tablespoons
31. Yogurt – all types	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
32. Ice cream, frozen yogurt, ice milk or milkshakes	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	125 ml or ½ cup
Grain Products				
33. Cereal – cold	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup

FOOD FREQUENCY QUESTIONNAIRE SECTION (continued)

A. FOOD		B. FREQUENCY	C. SERVING SIZE	
In the past month, did you consume:		How often did you consume it per day, week or month?	Using the average serving size as a guide, would your usual serving size be:	**Average Serving**
Grain Products **(continued)**				
34. Cereal – hot	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	175 ml or ¾ cup
35. White bread, rolls, buns, bagels, pita bread or tortillas	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	2 slices, rolls, buns, tortillas 1 bagel, pita
36. Whole grain bread *(whole wheat, oatmeal, rye, pumpernickel, etc.)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	2 slices
37. Pasta	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
38. Crackers	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	4 crackers
39. Cookies	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	2 cookies
40. Cake, muffins, pies, pastries, doughnuts	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 piece, muffin, pastry or doughnut
41. Cereal, muffin & oatmeal bars *(Nutri-Grain®, Hop & Go®, Oatmeal-To-Go®, etc.)*	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	1 bar
Other Foods				
42. Pizza	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	2 slices
43. Tea	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
44. Coffee	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	250 ml or 1 cup
45. Margarine	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	5 ml or 1 teaspoon
46. Butter	☐ Yes ☐ No	☐☐ times / ☐ day ☐ week ☐ month	☐ Less than average (small) ☐ Average (medium) ☐ More than average (large)	5 ml or 1 teaspoon

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 6 – Visit 6C - Lactational questionnaire *(between 3 and 8 weeks post delivery)*

HOME VISIT

Center | ID | Monogram

PROTOCOL DEVIATION SECTION

1. Were there any protocol deviations during this study visit? ☐ 1 Yes ☐ 0 No

If yes, specify

	Questionnaires 6A & 6B	**Specify Reason**
1.1 ☐	Questionnaire 6A not collected	1.1.1 _____
1.2 ☐	Questionnaire 6A not verified	1.2.1 _____
1.3 ☐	A part of the questionnaire 6A was not completed	1.3.1 _____
1.4 ☐	Milk collection sheet (6B) was not collected	1.4.1 _____
	Hair collection	
1.5 ☐	Problem with hair collection	1.5.1 _____
1.6 ☐	Problem with hair sample	1.6.1 _____
	Maternal milk sample	
1.7 ☐	Maternal milk sample not returned	1.7.1 _____
1.8 ☐	Quantity expected not reached	1.8.1 _____
1.9 ☐	Problem with transport and storage of the sample	1.9.1 _____
	Food frequency questionnaire	
1.10 ☐	A part of the questionnaire not completed	1.10.1 _____
	Other	
1.11 ☐	1.11.1 Specify _____	
1.12 ☐	1.12.1 Specify _____	

INTERVIEWER SECTION

1. Interviewer Initials ☐☐☐ 2. Duration of interview ☐☐☐ min

3. Signature _____ 4. Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

Reminder: Information regarding today's visit needs to be registered in the Follow-up log (Excel file)

Center	ID	Monogram

UNUSED ALIQUOT LABELS

☐ CHECK HERE IF ALL LABELS HAVE BEEN USED FOR THIS VISIT

If NOT, stick the unused label(s) into the corresponding box(es):

1. Hair

CC29 (bag)

Label code

124

2. Maternal milk

CC30 (glass jar)

Label code

125

CC31 (plastic jar)

Label code

126

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

Center	ID	Monogram	Day	Month	Year

1. Were there any protocol deviations during this participant follow-up? (visits not done, blood sample missing, freezing problem, etc.)

☐ 1 Yes ☐ 0 No

Investigator's statement

I have reviewed this woman's study data and confirm that, to the best of my knowledge, it accurately reflects the study information obtained for this woman.

Investigator's signature_____ 2. Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 8A – Delivery and post-partum questionnaire for multiple pregnancy

Centre | ID | Monogram | Day | Month | Year

BIRTH INFORMATION SECTION

1. BABY number: ☐

2. Delivery date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 3. Time (24 hr clock) ☐☐ hr ☐☐ min

CORD BLOOD SAMPLE COLLECTION SECTION

1. **Venous cord blood baby___:** Collection done? ☐ 1 Yes ☐ 0 No
   If no, complete the PROTOCOL DEVIATION SECTION

If yes

1.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 1.2 Time (24 hr clock) ☐☐ hr ☐☐ min

1.3 By: _____

1.4 Check containers collected If less containers than expected, complete the PROTOCOL DEVIATION SECTION

☐ 4.9ml SM (CC25_)

☐ Baxter bag (CC26_)

Note: Extract blood from the Baxter bag to fill out the 9x10ml tubes expected.

☐ 10ml EDTA (CC26.1__) ☐ 10ml EDTA (CC26.2__) ☐ 10ml EDTA (CC26.3__)
☐ 10ml EDTA (CC26.4__) ☐ 10ml EDTA (CC26.5__) ☐ 10ml EDTA (CC26.6__)
☐ 10ml EDTA (CC26.7__) ☐ 10ml EDTA (CC26.8__) ☐ 10ml EDTA (CC26.9__)

2. Processing of cord blood sample done? ☐ 1 Yes ☐ 0 No If yes 2.1 Time ☐☐ hr ☐☐ min
   If no, complete the PROTOCOL DEVIATION SECTION

3. Specimen frozen? ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If yes 3.1 Date of freezing ☐☐ ☐☐☐ ☐☐☐☐ 3.2 Time ☐☐ hr ☐☐ min

If delay between collection and freezing > 2 hours, complete the PROTOCOL DEVIATION SECTION

Reminder concerning Blood Specimen Tracking: Information above on specimen collection and storage needs to be included in the specimen log (Excel file)

MECONIUM SAMPLE COLLECTION SECTION

Important: the 2 x 10g of meconium can be collected in one or two occasions

1. Collection of 2 samples of 10 g of meconium done (1) 10g (CC27_) ☐ 1 Yes ☐ 0 No
   If 2 x 10g not collected, complete the PROTOCOL DEVIATION SECTION (2) 10g (CC28___) ☐ 1 Yes ☐ 0 No

If there was a collection, record the date and time of respective samples:

1.1 (CC27__) Date *dd mmm* ☐☐ ☐☐☐ 1.1.1 Time (24 hr clock) ☐☐ hr ☐☐ min

1.2 (CC28__) Date *dd mmm* ☐☐ ☐☐☐ 1.2.1 Time (24 hr clock) ☐☐ hr ☐☐ min

2. Freezing of meconium samples done ☐ 1 Yes ☐ 0 No If no, complete the PROTOCOL DEVIATION SECTION

If yes 2.1 Freezing date *dd mmm* ☐☐ ☐☐☐ 2.2 Time (24 hr clock) ☐☐ hr ☐☐ min

Reminder concerning Meconium Specimen Tracking: Information above on specimen collection and storage needs to be included in the Specimen Log (Excel file)

PROTOCOL DEVIATION SECTION

1. Were there any protocol deviations during this study visit? ☐ 1 Yes ☐ 0 No

If *yes*, specify:

		Specimen collection	**Specify Reason**
1.1	☐	Problem with meconium sample collection	1.1.1 _____
1.2	☐	Meconium specimen lost	1.2.1 _____
1.3	☐	Meconium freezing problem	1.3.1 _____
1.4	☐	Problem with cord blood collection	1.4.1 _____
1.5	☐	Cord blood tube lost	1.5.1 _____
1.6	☐	Cord blood processing problem	1.6.1 _____
1.7	☐	Cord blood freezing problem	1.7.1 _____

Other

1.8 ☐ 1.8.1 Specify _____

INTERVIEWER SECTION

1. Interviewer Initials ☐☐☐ 2. Duration of interview ☐☐☐ min

3. Signature _____ 4. Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

Reminder: Information regarding today's visit needs to be registered in the Follow-up log (Excel file)

UNUSED ALIQUOT LABELS

☐ CHECK HERE IF ALL LABELS HAVE BEEN USED FOR THIS VISIT

If NOT, stick the unused label(s) into the corresponding box(es):

Cord blood

CC25___(S-monovette)

Label code

96____(b or c, etc.)

CC26.1___(10mL EDTA)

Label code

97____(b or c, etc.)

CC26.2___(10mL EDTA)

Label code

101____(b or c, etc.)

Label code

103____(b or c, etc.)

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

UNUSED ALIQUOT LABELS (CONTINUED)

Cord blood (continued)

CC26.3___(10mL EDTA)

Label code

104____(b or c, etc.)

CC26.4___(10mL EDTA)

Label code

107____(b or c, etc.)

Label code

109____(b or c, etc.)

CC26.5___(10 mL EDTA)

Label code e

110____(b or c, etc.)

Label code

112____(b or c, etc.)

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

UNUSED ALIQUOT LABELS (CONTINUED)

Cord blood (continued)

CC26.6___(10mL EDTA)

Label code

113____(b or c, etc.)

Label code

115____(b or c, etc.)

CC26.7___(10mL EDTA)

CC26.8___(10mL EDTA)

CC26.9___(10mL EDTA)

Meconium

CC27___(10g)

CC28___(10g)

Before sending this CRF to the SCC: if there are one or more aliquot(s) missing, please make a copy of the corresponding page(s) and file in the patient's study file, to keep a record on site.

NOTE: the shaded squares refer to the BIOBANK SPECIMENS

MIREC Maternal-Infant Research on Environmental Chemicals

QUESTIONNAIRE 8B – Chart Review Questionnaire – Neonatal outcomes for multiple pregnancy

Centre | ID | Monogram | Day | Month | Year

NEONATAL INFORMATION SECTION baby____SECTION

1. Birthweight ____ g
2. Length at birth ____ • ____ cm
3. Cephalic perimeter ____ • ____ cm
4. Gender ____ 0 M ____ 1 F ____ 2 Unknown
5. Gestational age at delivery ____ weeks ____ days / 7
6. Placental weight ____ g
7. Did the baby have any congenital anomalies? ____ 1 Yes ____ 0 No

   If *yes* 7.1 Specify post-natal diagnosis _____

8. Was the baby born alive ____ 1 Yes ____ 0 No

   If *no* 8.1 Was there an autopsy performed? ____ 1 Yes ____ 0 No

   8.1.1 If yes, specify diagnosis based on autopsy _____

   8.1.2 If no autopsy, specify clinical diagnosis _____

   If the baby was not born alive, stop data collection here and go to the PROTOCOL DEVIATION SECTION

9. Apgar Score: 9.1 ____ 1 minute ____ Not done
   9.2 ____ 5 minutes ____ Not done
   9.3 ____ 10 minutes ____ Not done
10. Did the baby have convulsions? ____ 1 Yes ____ 0 No
11. Did the baby have respiratory distress? ____ 1 Yes ____ 0 No

    If *yes* 11.1 When was respiratory distress first noted:

    ____ 0 In the first 4 hours after birth ____ 1 Between 5-24 hours after birth ____ 2 More than 24 hours after birth

    11.2 Was oxygen therapy required? ____ 1 Yes ____ 0 No
12. Did the baby require assisted ventilation? ____ 1 Yes ____ 0 No If *yes* 12.1 For how long was the baby ventilated:

    ____ 0 Less than 24 hours ____ 1 24 hours to 7 days ____ 2 More than 7 days
13. Was the baby admitted to a neonatal intensive care unit? 1 Yes 0 ____ No

    If *yes* 13.1 Length of stay ____ day(s) ____ hours

    13.2 Diagnosis at admission _____
14. Was the baby admitted to an intermediate care unit? ____ 1 Yes ____ 0 No

    If *yes* 14.1 Length of stay ____ day(s) ____ hours

    14.2 Diagnosis at admission _____

NEONATAL INFORMATION SECTION baby____SECTION (continued)

15. Did the baby receive systemic antibiotics? ☐ 1 Yes ☐ 0 No

If *yes* 15.1 For what reason were they prescribed _____

16. Was a blood culture done? ☐ 1 Yes ☐ 0 No

If *yes* 16.1 Number of blood cultures done ☐☐

16.2 Culture result: ☐ 1 Positive ☐ 0 Negative 16.2.1 If positive, what species grew _____

17. Did the baby have an intraventricular hemorrhage? ☐ 1 Yes ☐ 0 No

If *yes* 17.1 Grade: ☐ **I** ☐ **II** ☐ **III** **IV** ☐

18. Did the baby have other intracranial hemorrhage? ☐ 1 Yes ☐ 0 No

If *yes* 18.1 Specify _____

19. Did the baby have necrotizing enterocolitis?	☐ 1 Yes	☐ 0 No
20. Did the baby have hypertonia?	☐ 1 Yes	☐ 0 No
21. Did the baby have retinopathy of prematurity?	☐ 1 Yes	☐ 0 No
22. Did the baby have leukomalacia?	☐ 1 Yes	☐ 0 No
23. Did the baby have neutropenia?	☐ 1 Yes	☐ 0 No
24. Did the baby die in the birth hospital?	☐ 1 Yes	☐ 0 No
If *yes* 24.1 Was there an autopsy performed?	☐ 1 Yes	☐ 0 No

24.1.1 If yes, specify diagnosis based on autopsy _____

24.1.2 If no autopsy, specify primary contributing cause _____

25. Other medical problem? ☐ 1 Yes ☐ 0 No

If *yes* 25.1 Specify _____

26. Was the baby transferred to another hospital ☐ 1 Yes ☐ 0 No

If *yes* 26.1 Name of the hospital _____

26.2 Reason of the transfer _____

27. Date and time of transfer/discharge from the birth hospital (or death):

27.1 Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐ 27.2. Time (24 hr clock) ☐☐ hr ☐☐ min

PROTOCOL DEVIATION SECTION

1. Was there any protocol deviation to this Chart review questionnaire ☐ 1 Yes ☐ 0 No

1.1 If *yes*, specify reason _____

INTERVIEWER SECTION

1. Interviewer Initials ☐☐☐ 2. Duration of review ☐☐☐ min

3. Signature _____ 4. Date *dd mmm yyyy* ☐☐ ☐☐☐ ☐☐☐☐

Reminder: Information regarding today's visit needs to be registered in the Follow-up log (Excel file)