Cotinine, Tobacco Smoke, and Diet: Strengthening our Understanding of this Biomarker in Early Life

by
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Abstract

There are concerns about the usefulness of cotinine and trans-3’-hydroxycotinine (3HC), as biomarkers of risk in populations with light tobacco smoke exposure. Using CHILD cohort study data, multiple linear and logistic regression was used to determine how well questionnaire responses explained urinary concentrations of cotinine and 3HC in infants, whether these concentrations predicted childhood asthmatic and allergic disease risk, and whether breastmilk facilitated nicotine exposure. Predictive models explained 31% and 41% of the variation in cotinine and 3HC, respectively. Only 23% of the infants had urinary concentrations consistent with second-hand smoke (SHS) exposure. The majority (92%) of household smoking occurred outdoors. While smokers breastfed less often, breastmilk did facilitate nicotine intake. The implications of dietary nicotine sources through breastmilk were inconclusive. Subclinical impacts and the pervasiveness of thirdhand smoke pose a challenge for public health and we should re-evaluate our use and interpretation of nicotine biomarkers in low-smoke exposure settings.

Keywords: second-hand smoke; thirdhand smoke; nicotine biomarkers
breastfeeding; smoking cessation
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<th>Description</th>
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<tr>
<td>MLR</td>
<td>Multivariable Linear Regression</td>
</tr>
<tr>
<td>RFR</td>
<td>Random Forest Regression</td>
</tr>
<tr>
<td>SHS</td>
<td>Second-hand Smoke</td>
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<tr>
<td>THS</td>
<td>Thirdhand Smoke</td>
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Chapter 1.

Introduction

1.1. Background

Tobacco smoke exposure has been studied at length for its negative health effects, particularly in children. Passive smoking exposure has been found to induce childhood asthma by affecting the balance of T-regulatory and T-helper cells (Jing, Wang, & Liu, 2019). Exposure to tobacco smoke during the prenatal period and infancy has long been of interest when predicting and preventing childhood asthma and wheeze (Silvestri, Franchi, Pistorio, Petecchia, & Rusconi, 2015). The recent ‘epidemic’ of childhood asthma in North America and other developed countries has been largely attributed to a change in our environmental exposures, with a particular interest in gene-environment interactions (Sears, 2014). Having tools and measures that properly assess and manage these exposures, such as biomarkers, validated questionnaires, and ambient exposures measures, has been a challenging and important task posed to scientists, clinicians, and decision-makers. Cotinine is perceived as the single best biomarker of tobacco smoke exposure (Centers for Disease Control and Prevention, 2016), often used by clinicians and researchers to make important decisions and interpretations about the health and risky behaviours of an individual or population. The fluidity of physical and social contexts is a challenge that pervades even the most renowned and objective measures of exposure. Heavily relied-upon measures must be validated and re-validated over time and across different populations in order to be helpful for clinical and policy purposes.

Biomarkers as a Tool for Exposure and Risk Assessment

When a cigarette is smoked, over 5000 components are released, many of which carcinogenic and toxic. However, only a fraction of these can be measured and interpreted for their risk based on the existing literature (Talhout et al., 2011). Carbon monoxide, ammonia, benzene, arsenic, cyanide, heavy metals, and a number of nitrogen oxides and free radical oxidants are some of these compounds (Talhout et al., 2011). While cigarette smoke is a carcinogen, nicotine itself is not. Instead, nicotine has been described as immunosuppressive and can dampen inflammatory responses.
through both humoral and cell-mediated mechanisms (Piao et al., 2009). Nicotine is an important component of cigarettes because it is highly addictive and can be objectively measured using biomarkers (Hukkanen, 2005). While it is understood that tobacco smoke carries many health risks, nicotine as the culprit for risk associated with exposure to tobacco smoke is controversial and incompletely characterized (Piao et al., 2009). When nicotine enters the body it is eventually metabolized, primarily by liver enzyme CYP2A6, into cotinine and trans-3'-hydroxycotinine which can then be detected in samples of urine, serum, hair, and saliva (Benowitz, Hukkanen, Jacob, & III, 2009) (Fig. 1.1). More than 70% of nicotine is metabolized by the liver enzyme CYP2A6 into cotinine, with most metabolism of cotinine results in trans-3'-hydroxycotinine (Benowitz et al., 2009; Dempsey et al., 2013). The lungs, spleen, kidneys and liver have the highest affinity for nicotine that travels through the bloodstream, and it is known to concentrate in amniotic fluid since it easily crosses the placental barrier, fetal serum, and breast milk (Benowitz et al., 2009).

The majority of smoking prevalence is currently held by high-income countries but smoking rates are dropping and tobacco companies are now targeting low and middle-income countries that have less tobacco control measures in place and who are experiencing economic upturns, meaning that the weight of tobacco-related morbidity is shifting from high to lower income countries (Lange, Probst, Rehm, & Popova, 2018). As a result, research in the past decade relating to tobacco smoke exposure has shifted from the health effects of first-hand smoking to that of second-hand smoking (SHS) risks in developed countries (Bird & Staines-Orozco, 2016; Zhou et al., 2014). In addition, the sensitivity of biomarker testing has increased, resulting in detection of lower concentrations and the ability to measure lower concentrations of nicotine metabolites. In populations characterized as having relatively low tobacco smoke exposure, the ability of urinary cotinine to predict clinical outcomes related to respiratory and allergic disease, known outcomes of tobacco smoke exposure, has been brought into question (Benowitz, Jain, Dempsey, Nardone, Helen, et al., 2017; Bramer & Kallungal, 2003). Some experts are now suggesting further investigation of whether dietary sources of nicotine, such as peppers and eggplant, can influence the levels of nicotine detected in children and infants, particularly in populations with a low prevalence of smoking (Hovell, Zakarian, Wahlgren, Matt, & Emmons, 2000).
Recent work has found that cotinine alone is a poor measure of one’s reported tobacco smoke exposure, particularly at lower levels (Benowitz, Jain, Dempsey, Nardone, St. Helen, et al., 2017; McLean, 2013; Torres et al., 2018). Despite this, some studies still use urinary cotinine biomarkers as the sole measure of tobacco smoke exposure. While cotinine and $\text{trans-3'}$-hydroxycotinine concentrations remain an objective way of verifying questionnaire reported tobacco smoking exposure, it appears to be less helpful or reliable in verifying little to no exposure.

**Building a Multifaceted Approach to Understanding the Context of Tobacco Smoke Exposure to Infants.**

Understanding who is exposed to tobacco smoke and how much they are exposed, what causes this exposure, when exposure occurs and how it changes over time, where they are most exposed, and why some are more exposed to tobacco smoke than others is key to deciding how to best measure, predict, and prevent this exposure. This thesis takes a multifaceted approach to understanding the current context of tobacco smoke exposure to Canadian infants using a combination of questionnaire, biomarker samples, and machine learning, and incorporates valuable health outcome and dietary data available from the CHILD Cohort Study.

![Nicotine Metabolism](image)

**Figure 1.1.  Nicotine Metabolism**  
Image by Jaclyn Parks. June 2020

**Significance of Thesis Findings**

As more sensitive tests with lower levels of detection have been put into practice, and the rates of smoking have dropped in Canada and other developed countries, little has been done to identify sources of low, but detectable levels of cotinine and rule out dietary nicotine as a potential source. If analysis shows that cotinine levels in the majority of our sample are unhelpful in clinical diagnosis, and that vegetable intake is significantly associated with cotinine concentrations amongst those with low tobacco exposure, the scientific application of cotinine as a biomarker of tobacco smoke will need
to change. This project will allow for a better understanding of how our biomarker data should be applied within the CHILD Study, and how nicotine metabolites should be considered and interpreted as biomarkers by future environmental health researchers.

1.2. Research Objectives, Study Population

The objectives of this thesis are as follows:

1) What are the levels and key sources of nicotine metabolites in a population of urban, Canadian infants?

2) Does diet contribute to low but detectable levels of cotinine in children? and

3) What are the clinical implications and relevance of these concentrations?

Specific research objectives are as follows:

• Using basic summary statistics and ANOVA to determine the levels of nicotine metabolites in infants and which predictors best explain differences in the concentration of these metabolites (Chapter 2).

• Use a combination of traditional model selection and machine learning to derive optimized models for predicting urinary metabolites of nicotine (Chapter 2).

• Determine how well a questionnaire-based prediction model can explain variation in urinary concentrations of cotinine and trans-3'-hydroxycotinine (Chapter 2).

• Discuss the research implications and the relevance of tobacco smoke exposure in this population to public health officials, researchers and clinicians (Chapter 2).

• Assess the relationship between nicotine metabolite concentrations in infants and respiratory and allergic health risks in childhood (Chapter 3).

• Explore whether infant diet and breastfeeding may help to predict variation in cotinine and 3HC concentrations amongst our study sample (Chapter 3).

• Determine if a maternal diet high in vegetables is associated with higher levels of cotinine in breastfeeding infants, and how this affects our understanding of nicotine metabolites as indicators of asthma risk for children (Chapter 3).
The CHILD Cohort Study

This MSc thesis will use data from the CHILD Cohort Study. The CHILD Cohort Study is the largest longitudinal cohort in Canada, with approximately 3,500 participants and their families followed from pregnancy until age 8, with intentions to follow subjects into adolescence. 3455 eligible children were recruited from largely-urban centers in 4 provinces across Canada (Vancouver, BC; Edmonton, AB; Winnipeg, Morden, and Winkler, MB, and Toronto, ON) to reflect the general Canadian, largely urban, population (Takaro et al., 2015). Using a comprehensive suite of exposure assessments, questionnaires, clinical measurements, and household and biomarker sampling, the CHILD Cohort study works primarily to understand how environmental exposures and genetics affect the risk of developing childhood asthma and allergies. Questionnaires relating to environmental exposures, psychosocial stresses, nutrition, and general health were completed by parents at recruitment in the second or third trimesters, and at ages 3, 6, 12, 18, 24, 30 and 36 months (Takaro et al., 2015). Periodic clinical assessments, skin prick tests, and biological sampling of subjects was also completed. The focus on inflammatory exposures in early life, as well as data on clinical and subclinical outcomes and dietary information makes the CHILD Cohort study the ideal population to address the objectives of this thesis.

1.3. Thesis Structure

This thesis consists of 2 papers suitable for peer-reviewed publication in the form of stand-alone chapters (Chapter 2, and 3) that each address one of the specific objectives outlined earlier, as well as a concluding chapter (Chapter 4).

Chapter 2 outlines the current state of smoking, exposure levels and sources of exposure to infants, and the limitations of strict use of biomarkers or questionnaire to understand the context of tobacco smoke exposure in infants. Concentration cut-offs for characterizing little to no smoke exposure to those with some second-hand smoke (SHS) exposure, and those with regular SHS exposure. Chapter 3 explores the relationship between breastfeeding, tobacco smoke exposure, and metabolized nicotine detected in infants. Chapter 4 concludes the thesis by highlighting the contribution of each paper and outlining the limitations of this work.
1.4. References


Chapter 2.

Assessing second-hand and third-hand tobacco smoke exposure in Canadian infants using questionnaires, biomarkers, and machine learning

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2.1. Abstract

Background

As smoking rates have dropped in Canada, the use of traditional tobacco smoke biomarkers in infants needs re-evaluation.

Objective

The objectives are to examine concentrations of nicotine biomarkers, their relationship with questionnaire responses to machine learning and prediction modeling to determine the extent and sources of tobacco smoke exposure for infants in urbanized Canadian families.

Methods

Questionnaire responses and infant urine samples from the CHILD cohort study were examined using multivariable linear regression models, chosen through a combination of conceptual and data-driven strategies including random forest regression to assess the ability of questionnaires to predict variation in biomarkers of tobacco smoke exposure: urinary cotinine and trans-3'-hydroxycotinine (3HC).

Results

Despite 2% of women in our sample smoking prior to and continuing through their pregnancy, we detected cotinine in the urine of 76% of the sample (n=2,017) and 3HC in 89%. Questionnaire-based models explained 31% and 41% of the adjusted $R^2$ variance in cotinine and 3HC levels, respectively. In addition to reported second-hand smoke exposure, housing characteristics, breastfeeding and reservoirs of third-hand smoke predicted nicotine metabolite concentrations. We identified general cut-points in cotinine and 3HC concentrations to characterize SHS exposure (0.25ng/mL and 0.50ng/mL).

Significance

Consistent with previous studies, prediction models for tobacco smoke exposure did not substantially explain much of the variation of cotinine and 3HC in a population with low but ubiquitous exposure. The use of these metabolites alone to measure tobacco smoke exposure remains problematic in this population. The ability of machine learning approaches to inform predictive modeling, the pervasiveness of thirdhand smoke
exposure, and the potential for non-smoke sourced nicotine to impact risk should be explored further.

### 2.2. Introduction

Tobacco smoke exposure has been studied at length for its negative health effects and is known to be particularly harmful to children (Chilmonczyk et al., 1993). Research in the past two decades relating to tobacco smoke exposure has shifted from the health effects of first-hand smoking to that of second-hand smoking (SHS) (Bird & Staines-Orozco, 2016; Lee et al., 2019; Zhou et al., 2014). SHS both in utero and in childhood has been repeatedly linked to asthma, as well as sudden infant death syndrome, low birth weight, cancer, dental caries, hearing loss and metabolic syndromes along with a breadth of poor behavioural and cognitive outcomes (Bruin, Gerstein, & Holloway, 2010; Zhou et al., 2014).

Accurately assessing prenatal and early life tobacco smoke exposure is important in understanding and preventing childhood asthma and wheeze (Silvestri, Franchi, Pistorio, Petecchia, & Rusconi, 2015). Questionnaires are a flexible and relatively inexpensive method of assessing exposure, but biomarkers of tobacco smoke exposure are more accurate, objective and can be obtained with little burden to the subject, such as in the case of passive urine sample collection. Nicotine is an important component of cigarettes for researchers because it can be detected in humans using biomarkers (Hukkanen, 2005). While there are many metabolites of nicotine that can be analyzed, cotinine is the most widely used biomarker of recent tobacco smoke exposure and has an average half-life of 16-19 hours in children ages 2 months to 4 years (Benowitz, Hukkanen, Jacob, & III, 2009; D. A. Dempsey et al., 2013). Cotinine, a metabolite of nicotine, is still heralded as the best biomarker of tobacco smoke exposure (Centers for Disease Control and Prevention, 2016). More than 70% of nicotine is metabolized into cotinine, with all metabolism of cotinine resulting in trans 3’-hydroxycotinine (3HC) (D. A. Dempsey et al., 2013). While cotinine and 3HC concentrations remain an unbiased way of verifying recent tobacco smoking exposure, they are less reliable in verifying little to no SHS exposure.

The environmental context in which cotinine has been used by researchers is changing. As tests with lower levels-of-detection have been put into practice, and
smoking rates have dropped in Canada and other developed countries, there are gaps in our knowledge of how these refinements may impact the use of this biomarker for public health, including identifying sources of low concentrations of nicotine. When smoking prevalence was higher, the use of cotinine to measure tobacco smoke exposure was warranted as it aided in verification of the confirmed exposure levels. However, over the past two decades the Canadian rates of smoking have been dropping, particularly during pregnancy and when around children. This is a combined result of policy, increased awareness, product labelling, clinician counseling and social pressures (Al-Sahab, Saqib, Hauser, & Tamim, 2010; Asbridge, 2004; Cawkwell, Lee, Shearston, Sherman, & Weitzman, 2016; Centers for Disease Control and Prevention, 2016; Hammond, Fong, & Mcdonald, 2003; Millar & Hill, 2004; Noar et al., 2016; Sánchez-Rodríguez et al., 2015). During the 1990’s, the rates of smoking during pregnancy were estimated to be 24% in Canada (Connor & McIntyre, 1999), which dropped to approximately 17% in 2000 (Millar & Hill, 2004), 11% in 2006 (Al-Sahab et al., 2010), and have continued to decline since.

The purpose of this study is to better understand cotinine and 3HC as biomarkers of tobacco smoked exposure in a population with little to no reported tobacco smoke exposure. The research questions are “What is the context of tobacco smoke exposure in a population of Canadian infants”, “What are the key sources of tobacco smoke exposure”, and “What are the research implications and the relevance of the context of smoke to policy-makers, researchers and clinicians?”. This project will allow for a better understanding of how our biomarker data should be organized within cohorts like the CHILD Study, and how low-level nicotine metabolites should be considered and interpreted as biomarkers by future environmental health researchers.

2.3. Materials/Participants and Methods

The cohort

This study uses of secondary data from the CHILD Cohort Study, a four centre (Vancouver, Edmonton, Winnipeg and Toronto) longitudinal, population-based birth-cohort study which enrolled 3,455 mother-child pairs between 2008 and 2012 with planned five-year follow-up. The main focus of CHILD is to identify environmental and genetic determinants of allergic disorders and asthma. The demographic characteristics
of the cohort are linked to reduced smoking rates relative to the general Canadian population, with a very small proportion of mothers in the study reporting that they smoked during pregnancy or during the child’s early life (McLean, 2013). Data was collected using a combination of questionnaires, in-home visits, and urine samples.

The urine samples

Urine samples were collected by trained research assistants during the 3-month in-home visits. The procedure involved placing a plastic Tegaderm™ film over the wetting area of the baby’s diaper to prevent urine absorption by the diaper. Cotton pads were placed on top of the film and the baby then wore the diaper for the duration of the home visit. At the end of the visit, the mother removed the diaper, and the research assistant placed the cotton pads into a syringe, aliquoted the sample into six vials, and measured the specific gravity of the sample using a calibrated refractometer. The samples were stored at -80 degrees Celsius (Takaro et al., 2015). In the laboratory, β-glucuronidase was used to deconjugate any glucuronidated cotinine and 3HC molecules. After extraction, the samples were analyzed by liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry (LC-APCI-MS). Analyte concentrations were calculated using least-squares linear regression of the peak area ratios of native to internal standards. The limit of detection for both cotinine and 3HC was 0.030 ng/mL.

To account for dilution of the urine samples, biomarker concentrations were corrected for specific gravity (McLean, 2013; Takaro et al., 2015). Samples with specific gravity (SG) measurements outside the normal human range (3 standard deviations above the median) were excluded from the analysis. Since the metabolite concentrations were approximately log-normally distributed, the concentrations were log-transformed (base 2) prior to SG correction. Concentrations below the 0.03 ng/mL level of detection were imputed using a truncated method (Lubin et al., 2004) (See Appendix). Concentrations were determined for each of the two metabolites separately, as well as on the sum of the two concentrations on a molar basis (picomole/mL).

Predictor variables

The exposure variables are taken from three sources; a questionnaire completed by the mother during pregnancy, a parent-completed household exposures questionnaire at 3 months, and a research assistant-completed questionnaire on household exposures
completed at the 3-month home visit. Potential predictors of exposure were derived from questionnaires which captured smoking-related exposure, housing characteristics, and demographics that have been linked in the literature to tobacco smoke exposure, be it second-hand, or third-hand (Tables 3a-c) (Benowitz, Dains, et al., 2009; Burton, 2011; Chen et al., 2016; Dahlström, Ebersjö, & Lundell, 2004; El-Mohandes, Kiely, Blake, Gantz, & El-Khorazaty, 2010; Hukkanen, 2005; Ray, Tyndale, & Lerman, 2009). Third-hand smoke results from second-hand smoke that has been absorbed onto surfaces such as carpeting and upholstery, or settled on dust where it can persist a long period of time and be released into the air long after a smoking event (Burton, 2011).

Geometric means and 95% confidence intervals of both cotinine and 3HC concentrations were calculated for the levels of each potential predictor variable. For normally distributed variables, we used t-tests or one-way analysis of variance (ANOVA) tests were used to assess whether the difference in means between levels or groups of a variable were statistically significant. When an ANOVA test was significant, Tukey Honest Significant Differences (Tukey-HSD) tests were run to assess multiple pairwise comparisons between multiple levels of a predictor. ANOVA and t-tests assume normality in the distribution of the means being compared. For non-normally distributed variable (determined using a Shapiro-Wilk test), a Wilcoxon test was used in place of a t-test, and a Kruskal-Wallis test in place of an ANOVA analysis. Spearman correlation tests were used when comparing the biomarker concentrations to a continuous predictor variable. The complete tables of geometric means by predictor and a dictionary of predictors considered are available in the appendix.

**Incorporation of Random Forest Regression in Prediction Model Selection**

A random forest regression (RFR) of all potential predictor variables (*a priori*) against each metabolite concentration assigned variable importance scores to each predictor. RFR is one example of a machine learning technique used to allow a system to automatically learn from the data and produce results with minimal subjective bias, offering an objective perspective on the variable selection process. The RFR was set to run models on 1000 trees, a relatively large number for RFR, to help ensure that the average model fit best reflects the data and avoids overfitting from using too few trees. These variable importance scores reflected the overall influence of the predictor in a prediction model, by showing how much the mean squared error (MSE) of a model
would increase as a result of the predictor being excluded from the model. The MSE is a measure of closeness of a fitted line to actual data points. MSE values are bounded between zero to infinity. The smaller the MSE, the closer the fit is to the data. Having a model with variables of high importance will result in a lower MSE.

Using variable importance scores derived from RFR, I created multivariable linear regression (MLR) models to predict urinary concentrations as the outcome. A MLR model was then selected based on questionnaire variables identified as ‘important’, and a-priori assessment to best explain the cotinine concentrations of the sample with detectable metabolite date. The models were built using manual selection, starting with the predictor with the highest variable importance score. The predictor with the next highest score was then added to the model. The added predictor was included if it had appropriate directionality, increased the model’s coefficient of determination ($R^2$), and was reasonably statistically significant ($p<0.15$). This was repeated for many predictor variables (see Appendix) until it was clear that adding more predictors did not further improve model performance. The coefficient of determination and measures of model fit were then used to determine how well questionnaire-based models explain variation in urinary cotinine concentrations in infants. This process was repeated for 3HC, as some variables may be more important for one metabolite than another. Plots of predicted versus observed biomarker concentrations were created for each final model to assess model fit. Coefficients and their 95% confidence intervals were reported for each predictor in the final MLR models against the log-transformed cotinine or 3HC concentration. These coefficients were then inverse-log-transformed to reflect the multiplicative change in urinary concentration (i.e. 1.10 means a 10% increase in concentration). The coefficients and back-transformations from unadjusted regression against the log-transformed urinary concentrations were also calculated for each predictor.

A 10-fold cross validation (CV) was also applied to the final trans-3’-hydroxycotinine and cotinine prediction models to evaluate prediction error for both models:

1. The dataset was randomly divided into 10 sub-groups with approximately the same number of observations in each group.
(2) The predictive model was parameterized based on data from 9 of the 10 groups.

(3) The estimated coefficients were used to predict log transformed urinary cotinine (or trans-3'-hydroxycotinine) concentrations for observations in the excluded group.

(4) Steps (1) – (3) were repeated to obtain predictions for all 10 groups and, therefore, all observations.

(5) Log transformed urinary concentration predictions and measurements on the untransformed scale were compared and model performance was evaluated based on $R^2$.

Characterizing exposure based on cut-points

Biomarkers can also be used to predict or verify the exposure level of participants. Density plots of the urinary metabolite concentrations by important questionnaire questions were used to examine the separation of the participants by questionnaire response (Dostál et al., 2008). We then compared density proportions of these responses within cut-point bounds to gain consensus about what average concentration is found in those who likely have no household exposure, those who have some light exposure, and those who have confirmed household second-hand smoke exposure. In some cases, these levels were continuous (eg. week of gestation that mother quit smoking), while others were factors (eg. Location) or ordinal factors (eg. household income). Recommended cut-points for urinary cotinine concentrations were assessed (Benowitz et al., 2017).

Statistical Software and Ethics

Analysis was completed using R version 3.5.1 (2018-07-02). Ethics approval was obtained through Simon Fraser University, deeming this project to be a minimal risk study [project number 2018s0608]. Research ethics approval for the overall CHILD study was obtained at each recruitment site and through the Hamilton Integrated Ethics Board (certificate number 07-2929).
2.4. Results

Of 2,017 infants with complete data (*Appendix A, Fig. 1*), 76% had detectable cotinine and 89% had detectable hydroxy-cotinine (3HC) levels. Participants from Manitoba (Winnipeg, Morden, and Winkler) made up the largest proportion of our sample (31%), followed by Vancouver (27%), Edmonton (22%), and Toronto (20%). More than half of our participants had a household income over $100,000/year (55%), lived in a single-family home (56%), and had a mother over age 30 (60%). 34% lived in a rented home, and 31% had at least one parent with asthmatic history of disease. Less than 3% reported actively smoking during their pregnancy, while 21% reported being recently exposed to smoke during pregnancy. 17.7% of mothers were smokers but had quit prior to their pregnancy. Of the 6% of our sample who did not quit prior to their pregnancy, 64% reported quitting during the pregnancy, leaving just 2% who continued to smoke throughout their pregnancy. 62% of mothers were exclusively breastfeeding their child, 26% were partially breastfeeding, and 12% reported not breastfeeding their child at 3-4 months of age. Only 12% reported that smoking had occurred at the home since the child’s birth, with the majority of household smoking occurring outdoors.

After correcting for urine dilution and imputing those below detection (*Appendix A*) the geometric mean cotinine concentration of our sample was 0.12 ng/mL (95% CI: 0.11-0.13), and geometric mean 3HC concentration was 0.22 ng/mL (95% CI: 0.21-0.24) and a combination of them both had a mean of 2.13 pmole/mL (95% CI; 1.98-2.28). The arithmetic mean (and Median) concentrations for cotinine were 1.87 ng/mL (Median 0.08 ng/mL), and 6.67 ng/mL (Median 0.16 ng/mL) for 3HC (*Table 2*).
Table 2.1. Demographic Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>% (N)</th>
<th>Geometric mean urinary Cotinine (95% CI), ng/mL</th>
<th>Geometric mean urinary trans-3’-Hydroxycotinine (95% CI), ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Study Centre</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancouver</td>
<td>26.7% (n=539)</td>
<td>0.10 (0.09-0.12)</td>
<td>0.16 (0.14-0.18)</td>
</tr>
<tr>
<td>Edmonton</td>
<td>19.9% (n=402)</td>
<td>0.13 (0.11-0.15)</td>
<td>0.28 (0.23-0.34)</td>
</tr>
<tr>
<td>Winnipeg, Morden, Winkler</td>
<td>30.9% (n=624)</td>
<td>0.14 (0.12-0.16)</td>
<td>0.29 (0.25-0.34)</td>
</tr>
<tr>
<td>Toronto</td>
<td>22.4% (n=452)</td>
<td>0.09 (0.08-0.10)</td>
<td>0.20 (0.17-0.22)</td>
</tr>
<tr>
<td><strong>Difference in means, p-value</strong></td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Household Income</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$0-49,999/year</td>
<td>10.0% (n=201)</td>
<td>0.26 (0.20-0.34)</td>
<td>0.55 (0.43-0.76)</td>
</tr>
<tr>
<td>$50,000-99,999/year</td>
<td>31.3% (n=631)</td>
<td>0.14 (0.12-0.16)</td>
<td>0.26 (0.22-0.29)</td>
</tr>
<tr>
<td>$100,000-149,999/year</td>
<td>26.4% (n=533)</td>
<td>0.09 (0.08-0.11)</td>
<td>0.19 (0.16-0.21)</td>
</tr>
<tr>
<td>$150,000+/year</td>
<td>23.3% (n=469)</td>
<td>0.08 (0.07-0.09)</td>
<td>0.14 (0.12-0.16)</td>
</tr>
<tr>
<td>Prefers to not say</td>
<td>9.1% (n=183)</td>
<td>0.12 (0.10-0.15)</td>
<td>0.28 (0.22-0.36)</td>
</tr>
<tr>
<td><strong>Difference in means, p-value</strong></td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Maternal Age at Enrolment</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 to 23 years old</td>
<td>3.8% (n=77)</td>
<td>0.42 (0.28-0.64)</td>
<td>1.01 (0.64-1.60)</td>
</tr>
<tr>
<td>24 to 30 years old</td>
<td>32.2% (n=649)</td>
<td>0.14 (0.12-0.16)</td>
<td>0.29 (0.25-0.33)</td>
</tr>
<tr>
<td>31 to 35 years old</td>
<td>41.9% (n=845)</td>
<td>0.10 (0.09-0.11)</td>
<td>0.19 (0.17-0.21)</td>
</tr>
<tr>
<td>36-46 years old</td>
<td>22.1% (n=446)</td>
<td>0.09 (0.08-0.11)</td>
<td>0.17 (0.15-0.20)</td>
</tr>
<tr>
<td><strong>Difference in means, p-value</strong></td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Characteristic</td>
<td>% (N)</td>
<td>Geometric mean urinary Cotinine (95% CI), ng/mL</td>
<td>Geometric mean urinary <em>trans-3’-Hydroxycotinine</em> (95% CI), ng/mL</td>
</tr>
<tr>
<td>------------------------</td>
<td>---------------</td>
<td>-----------------------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Child’s Sex</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>52.9% (n=1067)</td>
<td>0.11 (0.10-0.13)</td>
<td>0.20 (0.18-0.23)</td>
</tr>
<tr>
<td>Female</td>
<td>47.1% (n=950)</td>
<td>0.12 (0.10-0.13)</td>
<td>0.23 (0.20-0.26)</td>
</tr>
<tr>
<td>Difference in means, p-value</td>
<td>p=0.86</td>
<td>p=0.26</td>
<td></td>
</tr>
<tr>
<td><strong>Parental Asthma</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>31.3% (n=660)</td>
<td>0.13 (0.12-0.15)</td>
<td>0.24 (0.21-0.27)</td>
</tr>
<tr>
<td>No</td>
<td>67.3% (n=1357)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.22 (0.20-0.24)</td>
</tr>
<tr>
<td>Difference in means, p-value</td>
<td>p=0.03</td>
<td>p=0.11</td>
<td></td>
</tr>
<tr>
<td><strong>Rent vs. Own Home</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rent</td>
<td>23.2% (n=467)</td>
<td>0.19 (0.16-0.22)</td>
<td>0.36 (0.31-0.43)</td>
</tr>
<tr>
<td>Own</td>
<td>76.8% (n=1550)</td>
<td>0.10 (0.09-0.11)</td>
<td>0.19 (0.18-0.21)</td>
</tr>
<tr>
<td>Difference in means, p-value</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Dwelling Type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single Family</td>
<td>55.8% (n=1470)</td>
<td>0.10 (0.10-0.11)</td>
<td>0.20 (0.18-0.22)</td>
</tr>
<tr>
<td>Multi-Family or Apartment</td>
<td>25.8% (n=521)</td>
<td>0.15 (0.13-0.18)</td>
<td>0.29 (0.25-0.34)</td>
</tr>
<tr>
<td>Trailer or Other</td>
<td>1.2% (n=26)</td>
<td>0.24 (0.11-0.49)</td>
<td>0.53 (0.23-1.19)</td>
</tr>
<tr>
<td>Difference in means, p-value</td>
<td>p&lt;0.001</td>
<td>p&lt;0.001</td>
<td></td>
</tr>
<tr>
<td><strong>Breastfeeding Status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>12.0% (n=243)</td>
<td>0.16 (0.13-0.19)</td>
<td>0.29 (0.23-0.36)</td>
</tr>
<tr>
<td>Partial</td>
<td>25.8% (n=520)</td>
<td>0.12 (0.10-0.14)</td>
<td>0.24 (0.20-0.28)</td>
</tr>
<tr>
<td>Exclusive</td>
<td>62.2% (n=1254)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.23)</td>
</tr>
<tr>
<td>Difference in means, p-value</td>
<td>p=0.005</td>
<td>p=0.16</td>
<td></td>
</tr>
<tr>
<td>Characteristic</td>
<td>% (N)</td>
<td>Geometric mean urinary Cotinine (95% CI), ng/mL</td>
<td>Geometric mean urinary trans-3'-Hydroxycotinine (95% CI), ng/mL</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------------</td>
<td>----------------</td>
<td>-----------------------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Someone has smoked at the home since birth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No smoking at the home</td>
<td>87.8% (n=1771)</td>
<td>0.10 (0.09-0.10)</td>
<td>0.17 (0.16-0.19)</td>
</tr>
<tr>
<td>Yes, smoking at the home</td>
<td>12.2% (n=246)</td>
<td>0.50 (0.38-0.65)</td>
<td>1.36 (1.02-1.81)</td>
</tr>
<tr>
<td>Difference in means, p-value</td>
<td></td>
<td><strong>p&lt;0.001</strong></td>
<td><strong>p&lt;0.001</strong></td>
</tr>
<tr>
<td><strong>Location of household smoking during child's early life</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside</td>
<td>0.5% (n=10)</td>
<td>2.07 (0.79-5.44)</td>
<td>6.45 (2.50-16.63)</td>
</tr>
<tr>
<td>Near a Window or in Garage</td>
<td>1.6% (n=32)</td>
<td>1.30 (0.63-2.71)</td>
<td>4.18 (1.98-8.85)</td>
</tr>
<tr>
<td>Outside</td>
<td>11.2% (n=225)</td>
<td>0.43 (0.33-0.57)</td>
<td>1.16 (0.86-1.57)</td>
</tr>
<tr>
<td>Difference in means, p-value</td>
<td></td>
<td><strong>p&lt;0.001</strong></td>
<td><strong>p&lt;0.001</strong></td>
</tr>
<tr>
<td><strong>Mother reports smoke exposure during pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent Exposure</td>
<td>21.1% (n=425)</td>
<td>0.29 (0.24-0.35)</td>
<td>0.66 (0.54-0.81)</td>
</tr>
<tr>
<td>No recent exposures</td>
<td>78.9% (n=1592)</td>
<td>0.09 (0.08-0.10)</td>
<td>0.29 (0.24-0.35)</td>
</tr>
<tr>
<td>Difference in means, p-value</td>
<td></td>
<td><strong>p&lt;0.001</strong></td>
<td><strong>p&lt;0.001</strong></td>
</tr>
<tr>
<td><strong>Maternal smoking status in pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never Smoked</td>
<td>97.5% (n=1967)</td>
<td>0.10 (0.10-0.11)</td>
<td>0.20 (0.19-0.21)</td>
</tr>
<tr>
<td>Daily or Occasional Smoker</td>
<td>2.6% (n=50)</td>
<td>7.13 (4.18-12.14)</td>
<td>21.96 (12.21-39.48)</td>
</tr>
<tr>
<td>Difference in means, p-value</td>
<td></td>
<td><strong>p&lt;0.001</strong></td>
<td><strong>p&lt;0.001</strong></td>
</tr>
</tbody>
</table>

The proportion and crude number of sample subjects that corresponds to each level of household characteristic variables is reported to the nearest whole number. The geometric mean (95% Confidence interval) of the corrected and log-transformed Cotinine distribution for each level of each variable is also shown. P-values indicate whether the difference in log-transformed means was statistically significant (p<0.05) amongst the variable levels based on ANOVA tests.
Table 2.2. Summary statistics of each metabolite

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>10th %</th>
<th>25th %</th>
<th>50th %</th>
<th>Mean</th>
<th>75th %</th>
<th>90th %</th>
<th>SD</th>
<th>Geometric mean (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotinine*</td>
<td>0.02</td>
<td>0.04</td>
<td>0.08</td>
<td>1.87</td>
<td>0.23</td>
<td>0.77</td>
<td>13.73</td>
<td>0.12 (0.11-0.13)</td>
</tr>
<tr>
<td>3HC*</td>
<td>0.04</td>
<td>0.07</td>
<td>0.16</td>
<td>6.67</td>
<td>0.45</td>
<td>1.90</td>
<td>67.84</td>
<td>0.22 (0.21-0.24)</td>
</tr>
<tr>
<td>Molar Sum*</td>
<td>0.47</td>
<td>0.73</td>
<td>1.42</td>
<td>45.35</td>
<td>3.95</td>
<td>16.62</td>
<td>385.9</td>
<td>2.13 (1.98-2.28)</td>
</tr>
</tbody>
</table>

*Corrected for specific gravity and with concentrations imputed below the level of detection. Cotinine and 3HC are measured in units of ng/mL, while a combination of both are reported in picomole/mL as they were combined on a molar basis. SD = standard deviation; IQR = interquartile range.

Based on geometric mean concentrations (Appendix A) and density plots (Fig.2.1a-c), those who report no known exposure to tobacco smoke have a geometric mean urinary cotinine concentration of 0.09-0.12ng/mL, while those who report some exposure have more variable range, and those with reported second-hand smoke exposure have an average cotinine concentration of at least 0.25 ng/mL depending on the characterizing question. Applying cut-points of presumed exposure to our samples, based on threshold identified by Benowitz et al for a population of adolescents with low smoking exposure (Benowitz et al., 2017), we suspect that 1.6% (n=33) of our sample are actively exposed or exposed to recent heavy second-hand smoke (>=30ng/mL), 21.9% (n=441) were exposed to second-hand smoke (0.25-30ng/mL), and 76.5% (n=1,543) were exposed to light, third-hand smoke or none at all (<0.25 ng/mL). The same concentration thresholds did not apply well to 3HC concentrations, so thresholds of 0.25ng/mL and 30ng/mL were doubled (Figures 2.1a-c).

The selected MLR models predicted 31% of the log-transformed cotinine concentration using 13 predictors and 41% of the variation in the log-transformed 3HC concentration using 19 predictors (Figures 2.3a-b and 2.4a-b). 3HC may require more predictors because its higher concentrations are more sensitive to a breadth of exposure sources. 10-fold cross validation found that the models performed slightly poorer, at 30% and 37%, respectively. When the same model that was used to predict cotinine was used to predict the 3HC concentration, it explained less of the variation in 3HC than the 3HC-optimized model ($R^2$ 38.46%). Predictors from the cotinine model explained 35.1% of the variation in the log-transformed combined molar concentration, while predictors from the 3HC model were able to explain 36.5% of the combined concentration. 10-fold cross-validation found that the cotinine model explained 30%, and the 3HC model
explained 37% of the variation in the measured log-transformed combined molar concentration. The most important predictors appear to be whether or not the mother smoked and/or quit prior to the pregnancy, the number of cigarettes smoked at the home during the pregnancy, and whether someone had smoked at the home since the child’s birth, though all predictors added value to the model. Adjusted model coefficients show that those who had not quit prior to their pregnancy had an infant with twice the urinary cotinine concentration of mothers who never smoked, and mothers actively smoking during their pregnancy had an infant with more than 5 times the urinary cotinine concentration of non-smoking mothers.
Figure 2.1a. Density plots of log-transformed cotinine and 3HC concentrations based on maternal smoking status during pregnancy

Density plots show the distribution of the log-transformed cotinine (top) and trans-3'-hydroxycotinine (3HC) (bottom) concentrations based on response to "Did the mother quit smoking during the pregnancy?". Vertical lines indicate cut-offs of assumed exposure to tobacco smoke. The left group likely experienced very little to no SHS or THS, the middle had some light SHS, and those to the far-right had regular exposure to SHS. The dashed lines reflect 0.25 ng/mL and 30ng/mL, while dotted lines indicate 0.50 ng/mL and 60ng/mL.
Density plots of log-transformed cotinine and 3HC concentrations based on the number of daily cigarettes smoked at the home in early life

Density plots show the distribution of the log-transformed cotinine (top) and 3HC (bottom) concentrations based on response to “How many cigarettes (on average) are smoked at the home daily in the child’s early life?”. Vertical lines indicate cut-offs of assumed exposure to tobacco smoke. The left group likely experienced very little to no SHS or THS, the middle had some light SHS, and those to the far-right had regular exposure to SHS. The dashed lines reflect 0.25 ng/mL and 30ng/mL, while dotted lines indicate 0.50 ng/mL and 60ng/mL.
Density plots show the distribution of the log-transformed cotinine (top) and trans-3'-hydroxycotinine (bottom) concentrations based on response to "How many smokers lived at the home during pregnancy?". Density curves were not created for response categories which 2 or fewer subjects selected. Vertical lines indicate cut-offs of assumed exposure to tobacco smoke. The left group likely experienced very little to no SHS or THS, the middle had some light SHS, and those to the far-right had regular exposure to SHS. The dashed lines reflect 0.25 ng/mL and 30ng/mL, while dotted lines indicate 0.50 ng/mL and 60ng/mL.
Figure 2.3a. Cotinine Multivariable Linear Regression Model

Coefficients (point) and their 95% confidence intervals (line) are displayed for each variable in a model predicting log-transformed urinary cotinine concentration the prediction mode. Variables related to second-hand smoke are shown in red, not smoking-related variables in blue, and variables related to household characteristics in grey. Intervals with a point estimate displayed as a circle are based on bivariate analysis between each predictor and urinary cotinine, while estimates displayed with a triangle reflect estimates from multivariable model.
Figure 2.3b. Trans-3'-Hydroxycotinine Multivariable Linear Regression Model
Coefficients (point) and their 95% confidence intervals (line) are displayed for each variable in a model predicting log-transformed urinary trans-3'-hydroxycotinine concentration the prediction mode. Variables related to second-hand smoke are shown in red, not smoking-related variables in blue, and variables related to household characteristics in grey. Intervals with a point estimate displayed as a circle are based on bivariate analysis between each predictor and urinary trans-3'-hydroxycotinine, while estimates displayed with a triangle reflect estimates from multivariable model.
Figure 2.4a. Multiplicative Change in Cotinine Multivariable Linear Regression Model

Multiplicative change in urinary cotinine concentration (point) and 95% confidence intervals (line) are displayed for each variable in the prediction model calculated using the inverse-log-transformed coefficients. Variables related to second-hand smoke are shown in red, not smoking-related variables in blue, and variables related to household characteristics in grey. Intervals with a change estimate displayed as a circle are based on bivariate analysis between each predictor and urinary cotinine, while estimates displayed with a triangle reflect estimates from multivariable model.
Figure 2.4b. Multiplicative Change in trans-3’-Hydroxycotinine Multivariable Linear Regression Model

Multiplicative change in urinary trans-3’-hydroxycotinine concentration (point) and 95% confidence intervals (line) are displayed for each variable in the prediction model calculated using the inverse-log-transformed coefficients. Variables related to second-hand smoke are shown in red, not smoking-related variables in blue, and variables related to household characteristics in grey. Intervals with a change estimate displayed as a circle are based on bivariate analysis between each predictor and urinary trans-3’-hydroxycotinine, while estimates displayed with a triangle reflect estimates from multivariable model.
Figure 2.5. Measured vs. Predicted log-transformed urinary cotinine concentration

Relationship between predicted and measured log-transformed urinary metabolite concentrations for cotinine (top) and trans-3'-hydroxy-cotinine (bottom) based on their MLR models.
2.5. Discussion

Urinary nicotine metabolite concentrations

Nicotine exposure was nearly ubiquitous in our study population, with nearly 90% of the sample having some detectable level of nicotine metabolite(s). This is similar to a Korean cohort study in which 88% of non-smoking homes had infants with detectable concentrations of cotinine (Kim & Lee, 2016), and a study of American adolescents which found that nearly all participants were exposed to tobacco but that the majority of smoking exposure in this population was primarily from light second-hand smoke (SHS) and third-hand smoke (THS) exposure sources (Benowitz et al., 2017). Compared to similar studies (Becker et al., 1999; Benowitz et al., 2017; Georg E Matt et al., 1999; Olivieri et al., 2006), the urinary concentrations of nicotine metabolites in our study sample appear to be reflective of those with low or light SHS and/or THS exposure. 3HC had a larger interquartile range (0.38ng/mL vs. 0.19ng/mL) than cotinine, indicating that it varies more in the population and may be a more sensitive biomarker of nicotine exposure.

On average, the 3HC concentrations were at least twice that of the cotinine concentrations. This is likely due to the difference in the half-lives of nicotine and cotinine between infants and adults. Neonates and children under 1 year of age have lower nicotine metabolism rates, with a nicotine half-life three to four times longer than adults (Collier et al., 1994; D. Dempsey, Jacob, & Benowitz, 2000). The metabolism of cotinine into 3HC in neonates is similar to that of older children and adults (D. Dempsey et al., 2000). Part of this difference may be due to initially low hepatic blood flow in the transition from umbilical flow (Gow, Ghabrial, Smallwood, Morgan, & Ching, 2001), and the use of less efficient liver enzymes to metabolize nicotine in early life (Tateishi et al., 1997).

Prediction Models

Our study found that prediction models explained less than half of the variation in urinary biomarker concentrations. The predictive multiple linear regression models explained 31.43% of log-transformed cotinine, and 40.89% trans-3’-hydroxycotinine (3HC) concentrations in our cohort study. This is slightly lower than others have been able to predict of urinary cotinine (33-45%) in children, though these studies have children with
at least one household smoker which differs from the CHILD cohort (Georg E. Matt et al., 2000; Wong et al., 2002). Parental reports are helpful in characterizing smoke exposure, but less helpful when completed by non-smoking mothers (Georg E Matt et al., 1999). Others have had more success in predicting serum cotinine ($R^2$ of 61%) when including indoor air nicotine levels, duration of exposure and ventilation measures (Kalkbrenner et al., 2010). Plotted measured vs. predicted concentrations show some fanning of the observations, particularly at higher concentrations suggesting that the residuals have non-constant variance and that confidence intervals and significance tests should be interpreted with caution.

Our models may not better explain the variation in cotinine concentrations for a few reasons. The half-life of cotinine means that there will naturally be more variability for those with low or inconsistent exposure (Goniewicz et al., 2011). Another reason may be that our questionnaires are subject to reporting bias and cannot adequately detect all aspects of smoke exposure, namely thirdhand smoke (THS) exposure. Finally, those with detectable levels of cotinine and $trans$-$3'$-hydroxycotinine may be the result of nicotine exposures not related to tobacco smoke, such as diet (Siegmund, Leitner, & Pfannhauser, 1999).

While cotinine concentrations remain a reliable way of verifying questionnaire reported tobacco smoking exposure in populations with some level of SHS, it is unlikely that biomarkers or questionnaires adequately reflect true exposure on their own. Prediction models created to explain small-for-gestation size in a Chinese cohort of pregnant women found that self-reported smoking better predicted actual smoking exposure than cotinine measures (Xie et al., 2015). As researchers report lower levels of detection, nicotine exposure in many populations becomes nearly ubiquitous and may begin to lose its predictive power as a proxy of tobacco smoke-related health risks.

The model to predict 3HC contained more variables, with some of these additional variables related to carpeting, home ownership, and the season at collection of the urine samples. More influence due to suspected third-hand tobacco smoke reservoirs than was found in the cotinine model may exist because 3HC has a longer half-life and may be more sensitive to intermittent or low-level exposure sources. Important predictors to both models include tobacco smoke exposure to the mother during pregnancy and the presence of household smoking since the child’s birth.
Dwelling type, paternal education, income, and breastfeeding status were used in predicting both urinary cotinine and 3HC. Breastfeeding was likely a surrogate for other exposures related to tobacco exposure and explaining why those who were breastfed the least had the highest concentrations of urinary cotinine and 3HC. Breastfeeding went from being associated with lower concentrations to being associated with higher concentrations when adjusting for other smoking factors. In a prediction model where collinearity and correlated predictors are present, the coefficients become less reliably interpretable. Sparse data bias, a problem where coefficients for levels of a variable with relatively few participants, likely plays a role. For these reasons, assessing the geometric mean concentrations within our important predictors becomes more helpful than consideration of the adjusted model coefficients.

**Incorporation of Machine Learning**

Random forest analysis identified exposure items relating to breastfeeding, third-hand sources of tobacco smoke (eg. carpeting, area rugs), and household characteristics (eg. single-family detached vs. other buildings) as being particularly important in predicting the nicotine metabolite concentrations. The advantages of using machine learning methods in environmental epidemiology were recently described as an intelligent way to assess a magnitude of potential exposures and pathways (Saglani & Custovic, 2019). The benefit of blended multiple linear regression-random forest (MLR-RF) models (Yuchi et al., 2019) was recently illustrated in particulate air pollution and may be useful for assessing tobacco smoke pollution as well.

**Second-hand Smoke**

Prenatal smoking exposure and related behaviours were important predictors of early life cotinine and 3HC concentrations. 92% of mothers reported that they never smoked. Half of the remaining sample of mothers quit during pregnancy. However, one-fifth of mothers reported some recent exposure to tobacco smoke during their pregnancy, a proportion closer to the 24% of mothers who had smoked at some point before or during their pregnancy. 7.4% of the participants reported some tobacco smoke to their child at 3 to 4 months, and 4.4% of the sample reported that the baby had been exposed to tobacco smoke in the past week. Of the 183 parents that reported cigarettes smoked at the home daily in the child’s early life, 62% reported between 1 and 5 cigarettes smoked per day.
**Indicators of reduced exposure**

At households where smoking had occurred, it was predominantly reported that the smoking happened outdoors. This behaviour limits the extent and proximity of smoking to the child, and so reflects that parents have some understanding of the dangers of smoking around an infant (eg. smoking in the garage, or outside). While 12% of our sample reported that someone had smoked at the home since the child’s birth. 11% of the sample reported that smoking occurred outside of the house, and 1.6% reported that smoking occurs near a window or in the garage. Only 0.5% reported smoking occurred inside the home. By comparison, in pregnancy the same questions reported that 11% had a smoker living at the home, 9% had smoking occur outside the home, with 2% near a window or in the garage and 1% of the sample reporting smoking takes place inside the home. Although the proportion of smokers in the home is similar before and after birth, the location of where the smoking occurs changed slightly and supports the hypothesis that avoidance behaviour is greater in the presence of a child than in the presence of a pregnant woman. Taken together, these encouraging findings illustrate that most people who smoke at a home with an child or expectant mother will make the effort to smoke outside, reducing second-hand smoke exposure.

**Socioeconomic factors**

Household income, education level, and maternal age were inversely related with the child’s urinary concentrations. Younger mothers tend to have less formal education, and are less successful in quitting smoking (Connor & McIntyre, 1999). The dwelling type of the home was important in both prediction models. Those living in apartments or multi-family homes having higher urinary concentration of cotinine and 3HC. Second-hand smoke is a prominent issue for those in multi-family and multi-unit housing (Burton, 2011). Children living in rented homes (23%) had higher concentrations of urinary cotinine and 3HC than those who owned their home. Income, education, and housing are all interrelated factors that influence the likelihood of a child being exposed to tobacco smoke in their early life. The inclusion of these variables in prediction models are expected to add value because they capture smoke exposure not already captured by the questions directly related to SHS exposure. We hypothesize that younger, less educated and lower income mothers are more likely to have friends who smoke or visit public areas where smoking occurs, which may not be reflected in SHS-related questionnaire responses.
Third-hand Smoke

Third-hand smoke is a relatively novel concept, with the term first coined in the late 2000’s (Acuff, Fristoe, Hamblen, Smith, & Chen, 2016). Third-hand smoke (THS) occurs when second-hand smoke (SHS) interacts with the physical environment and is heavily adsorbed onto surfaces and accumulates in dust,(Burton, 2011). This contamination of surfaces and fabrics from SHS persists to be later released into the air. Third-hand smoke, or residual tobacco smoke pollutants, can be re-emitted back into a gas or can react with environmental oxidants or other pollutants to create secondary exposures (Acuff et al., 2016; Burton, 2011). Building type, and household furnishings may contribute to tobacco smoke exposure. Predictors related to household reservoirs of thirdhand smoke, such as carpeting, were important in modeling 3HC, though not cotinine. Carpeted flooring and area rugs in the home have been shown to harbour tobacco combustion products (G E Matt, 2004).

THS exposure can remain elevated for 6 months after smoking cessation, which is related to the number of reservoirs such as fabrics, carpets, and dust in the home (Békő et al., 2018; Georg E Matt et al., 2017). Upholstery, carpets, and other fabrics absorb the smoke more readily than other surfaces and can off-gas its contaminants over longer periods of time (Gee, Semple, Watson, & Crossfield, 2013; Leung, Ho, Wang, & Lam, 2018; Georg E Matt et al., 2017). Therefore, even after smoking cessation, a home may have residual exposure. Infant’s nicotine exposure was slightly higher during warmer seasons than in winter in our sample. This may be related to people spending more time outdoors or opening windows, potentially allowing the infiltration of cigarette smoke into the home. The use of cigarettes has been found to increase during warmer months when it is more pleasant of an experience to smoke outdoors, likely due to indoor air restrictions (Momperousse, Delnevo, & Lewis, 2007).

Characterizing and capturing THS remains a challenge to assessing tobacco smoke exposure. Questionnaires may not accurately capture the complex chemistry of combustion, furnishings, ventilation and human behavior. Third-hand exposure raises significant challenges for policy makers given the lack of human studies that consider this exposure (Burton, 2011; Jacob et al., 2017). Only in the past few years have researchers begun to tease out the effects and pervasiveness of thirdhand smoke (Hang et al., 2017; Leung et al., 2018; Northrup, Jacob, et al., 2016; Northrup, Matt, Hovell,
As a relatively new phenomena in public health, the public lacks awareness and understanding of third-hand smoke may be an important component of tobacco control (Díez-Izquierdo et al., 2018).

There appears to be a lack of understanding of the far-reaching effects of second-hand and thirdhand smoke. Some researchers have proposed that nearly 85% of tobacco smoke is invisible (Gee et al., 2013). While exposure may not be odorous or visible, these light exposures still carry risks (Burton, 2011), thereby posing a knowledge translation challenge for increasing public awareness. While 11-12% of the sample reported that someone in the home smoked, only 7.4% reported that any child had any exposure to smoking in early life. This is less than the 22% we estimated to have some SHS exposure. Only a quarter of those who reported a household smoker also reported that their baby had some level of smoking exposure. Similarly, of the 223 mothers who reported that a smoker lived at the home during pregnancy, just 49 (22%) reported being exposed to smoke while at their home on an occasional (n=25), or regular basis (n=24). While the majority of household smoking occurred outside, there is still the potential for exposure through ventilation and third-hand reservoirs. These inconsistencies may be due to social desirability bias, or the lack of awareness of the pervasiveness of second and thirdhand smoking.

**Quantifiably characterizing exposure**

Density plots (Figure 2.3) show that cut-points in urinary concentrations meant to characterize exposure are not perfect reflections of true exposure. Similar to findings by Dostal et al. (Dostál et al., 2008), there was notable overlap in the distribution of the infants' urinary concentrations by predictors meant to characterizing them as exposed or not. This makes the recommendation of cut-points for this population more difficult. We cautiously agree with the continued use of 0.25ng/mL of cotinine as a cut-point to differentiate those from some confirmed light SHS to those exposed to more intermittent or THS sources. For 3HC, recommend doubling the concentration used as cut-point in the distribution of cotinine. The use of the metabolites to characterize tobacco smoke exposure in infants of a population with relatively low exposure is challenged by the natural variability that comes with intermittent exposure, the half-life of these metabolites, and the potential for nicotine to be sourced from diet (Benowitz et al., 2017; Davis, Stiles, DeBethizy, & Reynolds, 1991) rather than tobacco smoke.
2.6. Limitations

We acknowledge that the participants in our study may not reflect the vulnerable population most at-risk for tobacco smoke exposure. While the low prevalence of tobacco smoke exposure in this cohort should be celebrated, this cohort does slightly underestimate true exposure experienced by the Canadian population. The parents of CHILD participants are more affluent, educated, and allergic or predisposed to sensitivity than the general Canadian population. Mothers who smoke during pregnancy are more likely to be of low socioeconomic status, non-immigrants, single, have a chronic disease, without a family doctor, and parenting without having attended prenatal classes (Al-Sahab et al., 2010; Cui, Shooshtari, Forget, Clara, & Cheung, 2014). As a result, our recommendations from this study can only be applied to populations similar to our cohort and may not be suitable for individuals from demographics linked to higher cigarette use.

At the time of data collection for questionnaires used by this study, e-cigarette use was not yet popularized, and marijuana use was still illegal in Canada. We recognize that we have been unable to assess for novel, non-cigarette tools for tobacco exposure, and encourage that future analysis make use of data on marijuana and e-cigarette use when characterizing tobacco smoke exposure.
2.7. Conclusion and Future Directions

Our results suggest that tobacco smoke questionnaire models may not accurately explain the majority of variation in cotinine nor trans-3'-hydroxycotinine concentrations within a population with relatively little smoking exposure. Questionnaires are a flexible and relatively inexpensive method of assessing exposure, but biomarkers of tobacco smoke exposure are considered as more accurate and can be obtained with little burden to the subject. However, cotinine alone is not a suitable measure of tobacco smoke exposure. Questions that best explained the variation in nicotine metabolite concentrations included whether the mother quit smoking prior to pregnancy, the number of cigarettes smoked daily at the home during pregnancy, and the presence of household smoking since the child’s birth. Tobacco smoke exposure models could use a combination of questionnaire and biomarker data to more accurately assess risk or consider other exposure assessment tools. Researchers need to be aware of the context of their sample population and be purposeful in the selection of the appropriate exposure measures. The ability of machine learning approaches, such as random forest regression to enhance modeling research makes it a new tool for exploring exposure in environmental health. While our smoking rates were low and parents appear to be motivated to avoid exposing the child to smoke, nicotine exposure was nearly ubiquitous.

As the Canadian population overall is reducing the rates of smoking, particularly during pregnancy and when around children – both as a result of policy and social pressures, our understanding of cotinine as a measure of smoking needs to change. With growing public health concern over e-cigarettes and marijuana smoking, this study highlights a lack of awareness of light second-hand and thirdhand smoke as an additional frontier of tobacco smoke research and action. Future work should focus on collaboration between qualitative and quantitative analysis to better understand the motivations behind reducing smoking exposure to children, cessation, and how the implications of second-hand smoke are understood by the public. The potential for dietary nicotine exposure to an infant, particularly in populations with low reported exposure, may help to predict urinary cotinine and 3HC concentrations and should be investigated.
2.8. Acknowledgements

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2.10. References


Chapter 3.

The relationship between infant’s urinary nicotine metabolites and childhood asthmatic disease; exploring implications of breastfeeding and diet.

Authorship

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3.1. Abstract

Introduction

Accurately assessing tobacco smoke exposure in early life is important to understanding and preventing childhood asthma. As levels of detection for nicotine metabolites, cotinine and trans-3'-hydroxycotinine (3HC) fall, re-examination of these markers in settings of light tobacco smoke exposure is needed. In addition to second and thirdhand smoke, breast milk provides an additional route of exposure to smoke and dietary sources of nicotine, complicating our interpretation of these biomarkers concentrations as risk measures.
Methods

Using data from 1,432 infants from the CHILD Cohort Study, urinary concentrations of cotinine and 3HC collected at 3-4 months of age were compared by breastfeeding, maternal diet, and a number of other factors in relation to reported tobacco smoke exposure. Logistic regression models were used to assess the relationship between urinary concentrations of cotinine and 3HC and multiple asthma-related health outcomes at 3 and 5 years of age. This relationship was compared to consideration of questionnaire responses to explain asthma and related risk. The ability for maternal diet high in vegetables to influence these infants' urinary cotinine and 3HC concentrations was assessed using bivariate and multivariable linear regression (MLR) models and likelihood ratio tests.

Results

Cotinine and 3HC levels were associated with a greater odds of recurrent wheeze at 1 and 5 years of age, but not helpful in predicting other outcomes related to allergic and asthmatic disease in childhood. The 62% of infants who were exclusively breastfed had the lowest prevalence of reported second-hand smoke exposure and the lowest urinary concentrations of cotinine and 3HC. MLR models found that exclusively breastfeeding only increased the infant’s nicotine exposure only if the mother smoked while she was pregnant. The presence of a household smoker was associated with higher concentrations of nicotine metabolites in infants, independent of the infant’s breastfeeding status. A maternal diet high in vegetables assumed to contain nicotine was not associated with their infant’s urinary cotinine nor 3HC concentrations.

Conclusions

When the mother is a smoker, breastfeeding increases nicotine exposure to the child. Breastfeeding does not appear to affect infant exposure when the mother is not a smoker, likely because the majority of household smoking occurs outdoors. Mothers that breastfeed should be encouraged to reduce or quit smoking and maintain breastfeeding rather than reduce or quit breastfeeding and continue smoking. Dietary sources of nicotine from the mother did not influence the child’s urinary concentrations or their risk of asthmatic disease.
3.2. Introduction

Assessment of early life exposures is important to understanding the gene-environment interactions that take place during this vulnerable window of development, and for the development of preventative measures to improve health outcomes. The problematic long-term health and economic burden has motivated investigations of environmental predictors of the illness to better diagnose, manage or predict asthma risk (Ismaila, Sayani, Marin, & Su, 2013; Subbarao et al., 2015). Comprehensive longitudinal cohort studies provide invaluable information about complex risk factors, pathways and exposure and/or developmental interactions that help to explain the development and persistence of asthma (Radhakrishnan et al., 2014; Sears, 2014).

Accurately assessing tobacco smoke exposure in early life is important in understanding and preventing the development of childhood asthma and wheeze (Silvestri, Franchi, Pistorio, Petecchia, & Rusconi, 2015). Questionnaires are a flexible and relatively inexpensive method of assessing exposure, but biomarkers of tobacco smoke exposure are regarded as more accurate measures and can be obtained with little burden to the subject. When a cigarette is smoked, over 5000 components are released, many of which are carcinogenic and toxic but only a fraction can be measured and linked with health outcomes (Talhout et al., 2011). Nicotine is a key component of tobacco smoke, which can be easily traced in human subjects using biomarkers. While there are many testable metabolites of nicotine, cotinine is the most widely used biomarker of tobacco smoke exposure. However, studies in recent years using tests with low levels of detection have found that while high concentrations of cotinine reliably predict second-hand smoke exposure, the ubiquity at low levels of exposure (typically reflecting light second-hand or thirdhand smoke) have not been clinically useful for predicting asthma risk (Benowitz et al., 2017).

Previous research has found that most subjects in the CHILD cohort study have very low tobacco smoke exposure, and that the majority of reported exposure to the infant is light second-hand smoke, or third-hand smoke (Chapter 2). Questionnaire-based predictive models have only been able to explain 31% and 41% of the variation of cotinine and trans-3'-hydroxycotinine concentrations (Chapter 2). The same predictive models found the infant’s breastfeeding status to be important to the fit of the models, warranting further investigation.
Clinical significance in low-exposure settings

A recent study found that cotinine is nearly ubiquitous in the urine of their adolescent population, and that the clinical significance of ‘light’ exposure (less than second-hand exposure) needs further research (Benowitz et al., 2017). It has been proposed by other studies that the clinical impacts of light second-hand or third-hand smoke are minimal but require further consideration (Northrup et al., 2016). Cotinine is a good way of distinguishing between active and passive smoke exposure (Goniewicz et al., 2011), but is less helpful in populations of infants where the only form of tobacco smoke exposure is passive second-hand and third-hand. Third-hand smoke occurs when second-hand smoke (SHS) adsorbed onto surfaces and reservoirs such as carpeting an upholstery, or accumulates in dust to be re-emitted over time, long after the initial smoking event (Burton, 2011). Tobacco smoke experts have suggested that the once-dismissed source of dietary nicotine through certain vegetables may now be relevant in populations with very little tobacco smoke exposure (Hovell et al., 2000). The benefit of cotinine as a marker of risk in a setting of light tobacco smoke exposure has been brought into question (Benowitz et al., 2017; Hovell, Zakarian, Wahlgren, Matt, & Emmons, 2000; Jacob et al., 2017). This work will analyze and discuss the use of urinary cotinine and 3HC to explain asthma risk and explain why this approach may be problematic in a population with relatively light tobacco smoke exposure. The work presented sources data from the Canadian Healthy Infant Longitudinal Development (CHILD) Study.

Implications of dietary sources of nicotine exposure

This study considers dietary sources of nicotine intake for infants, with implications both for breastfeeding recommendations as well as the use of common biomarkers in the context of low-level tobacco smoke exposure. People can be exposed to nicotine through inhalation, ingestion, and skin exposure. Tobacco plants produce nicotine naturally as a botanical insecticide (Benowitz, Hukkanen, Jacob, & III, 2009), and can also absorb it through contaminated soil and air (Selmar et al., 2015). Nicotine concentrations in these plants may differ by species and where their crop is grown, with nicotine often more concentrated in the skin of vegetables (Siegmund, Leitner, & Pfannhauser, 1999). If ingested, this circulating nicotine may be passed to a breastfeeding infant before it is further metabolized or excreted by the mother.
In health research, we tend to focus on the inhalation exposure route as the most prevalent as it relates to respiratory and cardiovascular disease, among others. Some studies have used cotinine as the sole measure of a subjects’ tobacco smoke exposure (Duby et al., 2015). This is a problematic approach when studying a population that may be exposed through ingestion and skin, and whose primary source of nicotine is not tobacco smoke but rather diet or household contact with reservoirs of smoke products (eg. carpeting). Nicotine can be passed from mother to child through breast milk (Becker et al., 1999), and that breastfeeding status helps to predict urinary cotinine and 3HC concentrations (Chapter 2). Though it is usually assumed that all nicotine passed through breast milk originates from maternal exposure to tobacco smoke, it is possible that some of this nicotine comes from diet or other exposures.

Nightshade vegetables such as eggplant, peppers, potatoes and tomatoes contain small but detectable levels of nicotine (Benowitz et al., 2017; Davis, Stiles, DeBethizy, & Reynolds, 1991; Sheen, 1988; Siegmund et al., 1999). The amount of nicotine that accumulates in the body from these dietary sources is comparable to that of a person exposed to tobacco smoke through third-hand exposure, with an estimated mean daily intake of 1.4 ug/day (Siegmund et al., 1999). One food toxicology study found tomatoes to have an average nicotine concentration of 7.3 ng/g, and potatoes had an average concentration of 15 ng/g wet weight (Davis et al., 1991).

Cotinine from dietary sources of nicotine are lower than concentrations measured in those with moderate second-hand smoke exposure, but the context of smoking exposure and cut-points used to measure smoking exposure with biomarkers has changed (Benowitz et al., 2009). If an infant’s urinary concentration of nicotine metabolites is the result of a maternal diet high in nicotine-containing vegetables, our understanding of these concentrations as an indicator of smoke-related asthma risk needs to change. The potential for this situation is relevant in populations where smoking exposure is low and vegetable access is relatively high and encouraged during pregnancy and a child’s early life.

Some researchers suggest that nicotine from diet is not clinically relevant and it will not confound interpretation of cotinine concentration as the sole measure of passive smoking exposure (Bramer & Kallungal, 2003; Repace, 1994). This argument may have been valid in a population primarily exposed to first-hand and heavy second-hand
exposure, the concept has not been explored with tests using very low levels of concentration detection or in a population with infrequent and low levels of tobacco smoke exposure. If nicotine metabolites are found to be sourced from vegetable intake, there is a potential for the anti-oxidant effects of vegetables to balance oxidizing effects of any tobacco smoke products and other stressors linked to asthma, minimizing or actually reducing the risk of asthma and allergic disease (Papadopoulou et al., 2015; Sordillo et al., 2019).

**Objectives**

The objectives of this project were to;

1) Assess the relationship between nicotine metabolite concentrations in infants and respiratory and allergic health risks in childhood.

2) Explore whether infant diet may help to predict variation in cotinine and 3HC concentrations amongst our study sample.

3) To determine if a maternal diet high in vegetables is associated with higher levels of cotinine in breastfeeding infants, and how this affects our understanding of nicotine metabolites as indicators of asthma risk for children.

**3.3. Methods**

**The Cohort**

This study made use of secondary data provided by the CHILD cohort study. Data was collected using a combination of questionnaires, clinical assessments (clinician visits; outcome phenotyping), home visits, and biological samples (urine samples). CHILD is the largest longitudinal birth cohort study in Canada, with 3455 children were recruited from largely-urban centers in 4 provinces across Canada (Vancouver, BC; Edmonton, AB; Winnipeg, Morden, and Winkler, MB; and Toronto, ON) that reflect the general urban Canadian population (Takaro et al., 2015). At age 5, over 90% of the original families are still enrolled in the study. CHILD is well-positions to add valuable knowledge to tobacco smoke exposure science for three main reasons: 1) The assay used is very sensitive with a relatively low level of detection (0.03ng/mL), 2) The population is young (participants recruited at birth, mean age of mothers is 32 years), and 3) There is a relatively low prevalence of reported smoking in the cohort (5% of mothers smoking before pregnancy, 3% continued to smoke during pregnancy). While the cohort is
ongoing, data used for this project was collected between 2008-2017. A dataset of 1,432 participants had complete data on our biomarkers of nicotine exposure, health outcomes, food frequency questionnaires, and other home environment and lifestyle questionnaires relating to tobacco smoke exposure (Appendix A: Sample Selection Flow Chart).

**Biomarker collection**

Urine samples were collected by trained research assistants at the 3-month follow-up home visits. The procedure for the collection of the samples has been previously outlined by the CHILD study (Takaro et al., 2015). Samples were then analyzed for cotinine and trans-3’-hydroxycotinine (3HC) at the Centers for Disease Control and Prevention's Tobacco Laboratory in Atlanta, GA. Samples were first hydrolyzed using β-glucuronidase to de-conjugate any glucoronidated cotinine and 3HC molecules. After extraction, the samples were analyzed by liquid chromatography-atmospheric pressure chemical ionization tandem mass spectrometry (LC-APCI-MS-MS) (Kato, Silva, Needham, & Calafat, 2005; Takaro et al., 2015). Analyte concentrations were calculated using least-squares linear regression of the peak area ratios of native to internal standards. The limit of detection for both cotinine and trans-3’-hydroxycotinine was 0.030 ng/mL. To account for dilution of the urine samples, biomarker concentrations were corrected for specific gravity measurements collected at the time of sample collection as previously described (Chapter 2). Concentrations below the level of detection were imputed using a truncated method (Lubin et al., 2004).

**Demographics, Tobacco Smoke, and Diet Variables**

Demographics and other variables relating to household or second-hand smoke exposure were determined using a combination of home environment questionnaires, home assessments, parent-completed health, socioeconomic and environment questionnaires.

Breastfeeding status was determined using a questionnaire completed when the child was approximately 3-4 months of age. Parents reported whether the child had exclusively been breastfed, partially breastfed, or never breastfed. A separate question gathered details of the infant's diet, distilled with 6 combinations of exposure breastmilk, formula, and the introduction of solid foods.
A food frequency questionnaire was given to mothers for completion during pregnancy (Fred Hutchinson Cancer Research Center, n.d.). Using these responses, a daily average vegetable intake score was calculated. Additionally, a prudent/healthy diet score was calculated using loading scores from principal components analysis that reflects how well a participant adhered to a specific type of diet, as previously described (de Souza et al., 2016). For those with a higher prudent/healthy diet score, vegetable intake was high and the intake of pizza and fried foods was very low. Nutritional epidemiology has moved beyond measuring intake of specific foods to using food pattern analysis, usually in the form of principle components analysis to improve estimates of dietary characterization in health research (Hu, 2002; Lioret et al., 2015; Robinson et al., 2007). The average frequency of nicotine intake was calculated based on existing literature on the nicotine content of certain foods, our food frequency questionnaires, and Health Canada portion size guidelines (Appendix B). Each of these dietary vegetable or nicotine intake during pregnancy variables were compared to the infant’s urinary cotinine and 3HC concentrations in early life.

**Modeling health outcomes**

For analysis of health outcomes at 1, 3 and 5 years of age, bivariate logistic regression models were used. CHILD has health outcome data from questionnaires completed by parents, questionnaires completed by clinicians at a clinical assessment, and results from skin prick tests for allergic sensitivity. The health outcomes considered in this study were atopy, recurrent wheeze, and asthma, with their meaning and characterization detailed below.

- **Atopy:** Atopy was determined by skin prick tests previously described for this cohort (Tran et al., 2018). The child had atopy if they had a 2mm wheal response to at least of the common allergens tested. Skin prick tests were completed at 1, 3, and 5 year clinical visits. Allergens tested at 1 year included 6 inhalant allergens (*Alternaria tenuis* fungus, cat hair, dog epithelium, house dust mites *Dermatophagoides pteronyssinus* and *D. farina*, and German cockroach), and four food allergens (whole cow’s milk, egg white, soybean, and peanuts). At 3 and 5 years of age, the same 4 food allergens, along with 13 inhalant allergens (*Alternaria tenuis*, cat hair, dog epithelium, house dust mites *Dermatophagoides pteronyssinus* and *D. farina*, grass, mid-west trees,
ragweed, weeds, cladosporium, penicillium, *Aspergillus fumigatus*, and German cockroach) were tested.

- **Recurrent Wheeze:** Recurrent wheeze has been defined by the CHILD study as “two or more episodes of wheeze in one year”, as derived by CHILD based on the follow-up questionnaire item: “If yes [to wheeze], how many episodes?”. The original question for the child health questionnaire was “In the last (specified time period), has your child had a wheezing noise (whistling sound) coming from his/her chest either with a cold or without a cold?”. An episode was defined as wheezing for at least 15 minutes at a time with episodes separated from each other by at least 7 days. Episodes that were within a week of each other were classified as one continuous episode. Wheezing episodes reported upon a clinical visit or reason for separate hospital visit were also considered.

- **Asthma Diagnosis:** While diagnosing a child with asthma at a young age is difficult and inconsistent over time, this outcome between three and five years of age is widely used by researchers. Asthma was defined using the questionnaire completed at each clinical assessment by a trained clinician. When asked “In your opinion, does this child have asthma?.”, they had response options of “No”, “Possible” and “Yes/Probable”. Those classified as “Possible” or “Probable” qualified as a suspected asthma case in this study.

Logistic regression models were used to assess the relationship between the log-transformed urinary concentrations in a continuous form against each of the health outcomes at 1, 3 and 5 years of age. The same analysis was then completed after stratifying the sample by breastfeeding status at 3-4 months of age (Exclusive, Partial, None). Subjects were then categorized by splitting the continuous distribution of each biomarker into two groups based on thresholds that reflect some second-hand smoke exposure (see Chapter 2). For cotinine, those at or above 0.25 ng/mL were considered to have some second-hand smoke exposure (Benowitz et al., 2017)(Chapter 2). Those with at least 0.50 ng/mL of 3HC were considered to have some level of second-hand smoke exposure. The logistic modeling process was then repeated using these binary exposure variables. To determine whether questionnaires do as good or better of a job at predicting asthma risk, the relationship between urinary cotinine and 3HC
concentrations and health outcomes was then compared to the relationship between questionnaire responses related to smoking and the same health outcomes. These questionnaire items pertained to whether the mother had quit smoking prior to pregnancy, whether household smoking had occurred since the child’s birth, and how many days the mother was exposed in past two weeks of pregnancy.

Exploring the link between breastfeeding and nicotine metabolite concentrations

Geometric mean concentration of cotinine and trans-3'-hydroxycotinine were calculated for the overall sample, as well as for each level of selected questionnaire-reported variables, such as breastfeeding. Analysis of variance (ANOVA) tests were used to determine whether the mean concentration of these biomarkers differed significantly by level of breastfeeding exposure to the infant at 3 months of age (exclusive, partial, or not breastfed). Linear regression models were used to determine the relationship between breastfeeding and urinary concentrations. These models were then adjusted for maternal smoking status in pregnancy, then by a variable reflecting whether or not a smoker lived at the infant’s home in early life. Regression coefficients and significance p-values were compared before and after adjustment to make interpretations about the true contribution of breastfeeding to an infant’s nicotine intake. Plots were also used to assess whether the child’s differing exposure by maternal smoking and household smoker presence was influenced by the child’s breastfeeding status.

Exploring the link between vegetable intake and nicotine metabolite concentrations in breastfed infants

Bivariate linear regression analysis was used to determine whether a high-vegetable maternal diet or a maternal ‘healthy’ diet score (de Souza et al., 2016) was associated with the infant’s urinary cotinine or 3HC concentration. This was completed using the overall sample, then within subsets based on breastfeeding status. We hypothesize that if a maternal diet that contains nicotine influences their infant’s exposure, it would be through breastmilk and therefore any such findings would be pronounced within the subset of infants who were exclusively breastfed.

These maternal diet variables were added to existing multivariable prediction models described in Chapter 2 to determine whether they added predictive value when explaining the variation in urinary concentrations of cotinine and 3HC. This was done for the entire sample, as well as for a subset of infants who had been exclusively breastfed.
in early life. Likelihood ratio tests were used, and the directionality and significance of these added variables in the full model were assessed.

Using food frequency questionnaire data, we were able to see at what frequency the mothers ate certain foods, some of which are expected to contain small amounts of nicotine. Using data on foods that have been found by previous studies (Davis et al., 1991; Sheen, 1988; Siegmund et al., 1999) to contain measurable amounts of nicotine, the weekly maternal intake of dietary nicotine was calculated for mothers in our sample of the CHILD study. These foods include raw tomatoes, tomato sauce, mashed potatoes, fried potatoes, ketchup, peppers, cauliflower and tea. The average reported nicotine concentration per gram (wet weight) was multiplied by the average number of grams in a single portion of that same food type, using portion sizes provided by Health Canada. This expected nicotine concentration per portion was then multiplied by the mothers reported portion size and by the number of times per week the mother reportedly at that food type. This continuous concentration (ng nicotine/week) of dietary nicotine in the mother was then analyzed against their infant’s urinary cotinine and 3HC concentration to determine whether an association existed. This analysis was completed for the overall sample, as well as a subsample of infants who had a urinary concentration of cotinine less than 0.25ng/mL, were exclusively breastfed, had mother who never smoked, and had no reported household smoking exposure in early life (n=555). This subset was of particular interest because it is at these low concentrations with no reported smoke exposure and active breastfeeding that dietary sources of nicotine may become relevant in contributing to an infant’s nicotine metabolite concentration.

Analysis was completed using RStudio (R version 3.5.1 (2018-07-02)). Ethics approval was obtained through Simon Fraser University, deeming this project to be a minimal risk study (project number 2018s0608).

3.4. Results

Sample demographics

Of our final sample size of 1,432 (see Appendix B), 75% had detectable cotinine and 89% had detectable trans-3’-hydroxycotinine (3HC) levels. After correcting for urine dilution and imputing those below the limit of detection, geometric mean concentration of cotinine was 0.11 ng/mL (95% CI; 0.11-0.12), while 3HC had a geometric mean of 0.21
ng/mL (95% CI; 0.20-0.23) (Table 3.1). Using 0.25ng/mL and 0.50 ng/mL as thresholds of cotinine and 3HC to characterize a participant as having some second-hand smoke (SHS) or thirdhand smoke (THS), 22-23% of our sample likely had some SHS or THS exposure (n=325 for Cotinine and 320 for 3HC).

Table 3.1. Summary statistics of each metabolite

<table>
<thead>
<tr>
<th>Metabolite</th>
<th>10th %</th>
<th>25th %</th>
<th>Median</th>
<th>Mean</th>
<th>75th %</th>
<th>90th %</th>
<th>SD</th>
<th>Geometric mean</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotinine*</td>
<td>0.02</td>
<td>0.04</td>
<td>0.08</td>
<td>1.99</td>
<td>0.22</td>
<td>0.75</td>
<td>15.40</td>
<td>0.11 (0.11-0.12)</td>
</tr>
<tr>
<td>3HC*</td>
<td>0.04</td>
<td>0.07</td>
<td>0.15</td>
<td>7.30</td>
<td>0.43</td>
<td>1.66</td>
<td>78.16</td>
<td>0.21 (0.20-0.23)</td>
</tr>
</tbody>
</table>

*Corrected for specific gravity and with concentrations imputed below the level of detection. Cotinine and 3HC are measured in units of ng/mL. % = percentile of distribution, SD=standard deviation.

In our sample (n=1,432), more than half had a household income over $100,000/year, lived in a single-family home, 21% lived in a rented home, and 34% had at least one parent with a history or current diagnosis of asthma. More than 60% of mothers in our sample were over 30 years of age (mean age of 32 years), 5% were smokers before their pregnancy, 3% reported some smoking during their pregnancy and less than 2% reported actively smoking during their pregnancy. 62% of mothers were exclusively breastfeeding their child at 3 months of age, while 26% were partially breastfeeding, and 12% reported never breastfeeding their child. Of those who were partially breastfeeding their child, the majority were incorporating formula into the child's diet. Only 11% reported that smoking had occurred at the home since the child's birth, most of this household smoking occurring outdoors. Participants from Manitoba (Winnipeg, Morden, and Winkler) made up the largest proportion of our sample (35%), followed by Vancouver (30%), Edmonton (19%), and Toronto (17%).


<table>
<thead>
<tr>
<th>Characteristic</th>
<th>% (N)</th>
<th>Geometric mean urinary Cotinine (95% CI), ng/mL</th>
<th>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Breastfeeding Status</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>12.1 (173)</td>
<td>0.16 (0.12-0.20)</td>
<td>0.28 (0.21-0.37)</td>
</tr>
<tr>
<td>Partial</td>
<td>25.8 (369)</td>
<td>0.11 (0.09-0.13)</td>
<td>0.22 (0.18-0.26)</td>
</tr>
<tr>
<td>Exclusive</td>
<td>62.2 (890)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.20 (0.18-0.22)</td>
</tr>
<tr>
<td><strong>Infant’s 3-month Diet</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breastfeeding Only</td>
<td>62.2% (890)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.20 (0.18-0.22)</td>
</tr>
<tr>
<td>Breastfeeding and Formula</td>
<td>24.2% (344)</td>
<td>0.11 (0.09-0.13)</td>
<td>0.22 (0.18-0.27)</td>
</tr>
<tr>
<td>Breastfeeding and Solid Food</td>
<td>0.9% (n=13)</td>
<td>0.08 (0.03-0.21)</td>
<td>0.20 (0.07-0.57)</td>
</tr>
<tr>
<td>Formula Only</td>
<td>11.2% (n=163)</td>
<td>0.15 (0.12-0.20)</td>
<td>0.27 (0.20-0.36)</td>
</tr>
<tr>
<td>Formula and Solid Food</td>
<td>0.8% (n=10)</td>
<td>0.19 (0.05-0.76)</td>
<td>0.47 (0.10-2.12)</td>
</tr>
<tr>
<td>Breastfeeding, Formula, and Solid Food</td>
<td>0.6% (n=12)</td>
<td>0.12 (0.05-0.30)</td>
<td>0.23 (0.07-0.78)</td>
</tr>
<tr>
<td><strong>Centre</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancouver</td>
<td>29.6 (424)</td>
<td>0.11 (0.09-0.13)</td>
<td>0.15 (0.13-0.18)</td>
</tr>
<tr>
<td>Edmonton</td>
<td>19.1 (274)</td>
<td>0.13 (0.11-0.16)</td>
<td>0.28 (0.22-0.35)</td>
</tr>
<tr>
<td>Winnipeg, Morden, and Winkler</td>
<td>34.7 (497)</td>
<td>0.12 (0.11-0.14)</td>
<td>0.25 (0.22-0.29)</td>
</tr>
<tr>
<td>Toronto</td>
<td>16.6 (237)</td>
<td>0.09 (0.08-0.11)</td>
<td>0.21 (0.17-0.25)</td>
</tr>
<tr>
<td><strong>Household Income</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$0-49,999/year</td>
<td>9.2 (132)</td>
<td>0.26 (0.19-0.36)</td>
<td>0.51 (0.36-0.71)</td>
</tr>
<tr>
<td>$50,000-99,999/year</td>
<td>32.3 (463)</td>
<td>0.14 (0.12-0.16)</td>
<td>0.24 (0.21-0.28)</td>
</tr>
<tr>
<td>$100,000-149,999/year</td>
<td>27.7 (396)</td>
<td>0.10 (0.08-0.11)</td>
<td>0.19 (0.16-0.22)</td>
</tr>
<tr>
<td>$150,000+/year</td>
<td>21.9 (314)</td>
<td>0.08 (0.07-0.09)</td>
<td>0.13 (0.11-0.15)</td>
</tr>
<tr>
<td>Prefers to not say</td>
<td>8.9 (127)</td>
<td>0.12 (0.09-0.15)</td>
<td>0.28 (0.21-0.39)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>% (N)</td>
<td>Geometric mean urinary Cotinine (95% CI), ng/mL</td>
<td>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</td>
</tr>
<tr>
<td>--------------------------------------</td>
<td>-------</td>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td>Maternal Age at Enrolment</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 to 23 years old</td>
<td>3.3 (47)</td>
<td>0.40 (0.25-0.65)</td>
<td>0.83 (0.46-1.47)</td>
</tr>
<tr>
<td>24 to 30 years old</td>
<td>33.5 (480)</td>
<td>0.14 (0.12-0.16)</td>
<td>0.27 (0.23-0.31)</td>
</tr>
<tr>
<td>31 to 35 years old</td>
<td>41.3 (592)</td>
<td>0.10 (0.09-0.11)</td>
<td>0.18 (0.16-0.21)</td>
</tr>
<tr>
<td>36-46 years old</td>
<td>21.9 (313)</td>
<td>0.09 (0.08-0.11)</td>
<td>0.17 (0.15-0.21)</td>
</tr>
<tr>
<td>Child's Sex</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>53.1 (760)</td>
<td>0.11 (0.10-0.13)</td>
<td>0.20 (0.18-0.23)</td>
</tr>
<tr>
<td>Female</td>
<td>46.9 (672)</td>
<td>0.12 (0.10-0.13)</td>
<td>0.23 (0.20-0.26)</td>
</tr>
<tr>
<td>Parental Asthma</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>66.0 (945)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.23)</td>
</tr>
<tr>
<td>Yes</td>
<td>34.0 (487)</td>
<td>0.13 (0.11-0.14)</td>
<td>0.22 (0.19-0.26)</td>
</tr>
<tr>
<td>Home Ownership</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rents</td>
<td>21.4 (307)</td>
<td>0.19 (0.16-0.23)</td>
<td>0.34 (0.28-0.42)</td>
</tr>
<tr>
<td>Owns</td>
<td>78.6 (1125)</td>
<td>0.10 (0.09-0.11)</td>
<td>0.19 (0.17-0.21)</td>
</tr>
<tr>
<td>Household Smoker, Early Life</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11.4 (163)</td>
<td>0.47 (0.33-0.65)</td>
<td>1.28 (0.89-1.84)</td>
</tr>
<tr>
<td>No</td>
<td>88.6 (1269)</td>
<td>0.10 (0.09-0.10)</td>
<td>0.17 (0.16-0.18)</td>
</tr>
<tr>
<td>Where smoking occurs at the home in early life</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outside vs. not</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Near Window vs. not</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Indoors vs. not</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Outside</td>
<td>10.7 (153)</td>
<td>0.43 (0.30-0.60)</td>
<td>1.19 (0.81-1.73)</td>
</tr>
<tr>
<td>Near window or in garage</td>
<td>1.3 (18)</td>
<td>1.09 (0.39-3.01)</td>
<td>3.22 (1.04-9.98)</td>
</tr>
<tr>
<td>Indoors</td>
<td>0.4 (6)</td>
<td>1.51 (0.31-7.31)</td>
<td>4.10 (0.98-17.12)</td>
</tr>
<tr>
<td>Mother's Smoking Frequency in Pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Daily or Occasionally</td>
<td>1.5 (22)</td>
<td>10.44 (4.52-24.14)</td>
<td>31.53 (11.73-84.75)</td>
</tr>
<tr>
<td>Never</td>
<td>98.5 (1410)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.20 (0.18-0.21)</td>
</tr>
</tbody>
</table>

The proportion and crude number of sample subjects that corresponds to each level of household characteristic variables is reported to the nearest whole number. The geometric mean (95% Confidence interval) of the corrected and...
log-transformed Cotinine distribution for each level of each variable is also shown. P-values indicate whether the difference in log-transformed means was statistically significant (p<0.05) amongst the variable levels based on ANOVA tests.

Table 3.3. Geometric mean cotinine and trans-3'-hydroxycotinine concentrations by asthma, atopy, and recurrent wheeze outcomes at 1, 3 and 5 years of age

<table>
<thead>
<tr>
<th>Health Status</th>
<th>Cotinine GM* (95% CI)</th>
<th>3'-Hydroxycotinine GM* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>1 year of age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Recurrent Wheeze (n=1319, 92.1%)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.23)</td>
</tr>
<tr>
<td>Recurrent Wheeze (n=113, 8.3%)</td>
<td>0.18 (0.12-0.25)</td>
<td>0.31 (0.22-0.47)</td>
</tr>
<tr>
<td>No Atopy (n=1244, 86.9%)</td>
<td>0.12 (0.11-0.13)</td>
<td>0.22 (0.20-0.25)</td>
</tr>
<tr>
<td>Atopy (n=188, 13.1%)</td>
<td>0.09 (0.07-0.12)</td>
<td>0.16 (0.13-0.20)</td>
</tr>
<tr>
<td>No Asthma (n=1425, 99.5%)</td>
<td>0.11 (0.11-0.12)</td>
<td>0.21 (0.20-0.23)</td>
</tr>
<tr>
<td>Asthma (n=7, 0.5%)</td>
<td>0.11 (0.03-0.40)</td>
<td>0.16 (0.04-0.74)</td>
</tr>
<tr>
<td><strong>3 years of age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Recurrent Wheeze (n=1309, 91.4%)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.23)</td>
</tr>
<tr>
<td>Recurrent Wheeze (n=123, 8.6%)</td>
<td>0.13 (0.09-0.18)</td>
<td>0.25 (0.18-0.35)</td>
</tr>
<tr>
<td>No Atopy (n=1214, 84.8%)</td>
<td>0.12 (0.11-0.13)</td>
<td>0.22 (0.20-0.25)</td>
</tr>
<tr>
<td>Atopy (n=218, 15.2%)</td>
<td>0.10 (0.08-0.12)</td>
<td>0.17 (0.14-0.21)</td>
</tr>
<tr>
<td>No Asthma (n=1255, 87.6%)</td>
<td>0.11 (0.10-0.13)</td>
<td>0.21 (0.20-0.24)</td>
</tr>
<tr>
<td>Asthma (n=177, 12.4%)</td>
<td>0.11 (0.09-0.14)</td>
<td>0.21 (0.17-0.27)</td>
</tr>
<tr>
<td><strong>5 years of age</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Recurrent Wheeze (n=1336, 93.2%)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.23)</td>
</tr>
<tr>
<td>Recurrent Wheeze (n=96, 6.7%)</td>
<td>0.17 (0.12-0.24)</td>
<td>0.31 (0.21-0.48)</td>
</tr>
<tr>
<td>No Atopy (n=1151, 80.4%)</td>
<td>0.12 (0.11-0.13)</td>
<td>0.22 (0.20-0.25)</td>
</tr>
<tr>
<td>Atopy (n=281, 19.6%)</td>
<td>0.10 (0.09-0.12)</td>
<td>0.18 (0.15-0.22)</td>
</tr>
<tr>
<td>No Asthma (n=1211, 84.6%)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.23)</td>
</tr>
<tr>
<td>Asthma (n=221, 15.4%)</td>
<td>0.13 (0.10-0.16)</td>
<td>0.25 (0.20-0.33)</td>
</tr>
</tbody>
</table>

Geometric mean concentrations of infant’s urinary cotinine and trans-3'-hydroxycotinine are reported based on their health status as later characterized at 1, 3, and 5 years of age.
Modeling childhood asthmatic disease

Of our sample of 1,432 participants, 325 infants (22%) were considered to be SHS/THS-exposed based on their cotinine concentration, while 320 (23%) were considered to be SHS/THS-exposed based on their 3HC concentration. The proportion of participants who developed recurrent wheeze was 8.3% (n=113) at one year, 8.6% (n=123) at 3 years, and 6.7% (n=96) at 5 years of age. The proportion of participants with atopy was 13.1% (n=188) at one year, 15.2% (n=218) at 3 years, and 19.6% (n=281) at 5 years of age. The proportion of participants with a possible or probable asthma diagnosis at one year of age was 0.5% (n=7). At 3 years of age, 6.7% (n=96) of participants had possible and 5.7% (81) had probable asthma. At 5 years of age, 7.9% (n=113) of participants had possible and 7.5% (n=108) had probable asthma.

Bivariate logistic regression analysis found that log-transformed cotinine and 3HC concentrations were associated with recurrent wheeze at 1 and 5 years, but not at 3 years (Table 3.3). Nicotine metabolite concentrations were negatively associated with atopy at 1 and 3 years of age, but not at 5 years of age (Table 3.3). No significant associations were found between cotinine nor 3HC and a possible or probable asthma diagnosis, whether these were measured as continuous or categorical variables (Table 3.4).
Table 3.4. Cotinine and *trans*-3'-hydroxycotinine as a continuous predictor of childhood asthma & intermediates at 1, 3 and 5 years of age vs. questionnaire-derived smoking predictors

<table>
<thead>
<tr>
<th></th>
<th>Recurrent Wheeze OR (95% CI), p-value</th>
<th>Atopy OR (95% CI), p-value</th>
<th>Asthma Diagnosis OR (95% CI), p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Health Outcomes at 1 year of age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotinine</td>
<td>1.11 (1.04-1.19), ( p=0.002 )</td>
<td>0.93 (0.86-1.00), ( p=0.05 )</td>
<td>-</td>
</tr>
<tr>
<td><em>Trans</em>-3'-hydroxycotinine</td>
<td>1.09 (1.02-1.16), ( p=0.01 )</td>
<td>0.92 (0.85-0.98), ( p=0.02 )</td>
<td>-</td>
</tr>
<tr>
<td>Mother quit smoking prior to pregnancy vs. Never Smoked</td>
<td>0.98 (0.58-1.58), ( p=0.94 )</td>
<td>0.72 (0.46-1.08), ( p=0.13 )</td>
<td>-</td>
</tr>
<tr>
<td>Mother smoked into pregnancy vs. Never Smoked</td>
<td>2.08 (1.01-3.96), ( p=0.03 )</td>
<td>0.66 (0.27-1.37), ( p=0.31 )</td>
<td>-</td>
</tr>
<tr>
<td>Any household smoking in early life vs. None</td>
<td>1.89 (1.12-3.04), ( p=0.01 )</td>
<td>0.75 (0.43-1.23), ( p=0.28 )</td>
<td>-</td>
</tr>
<tr>
<td>Days mom was exposed in past two weeks of pregnancy</td>
<td>1.05 (0.99-1.10), ( p=0.08 )</td>
<td>0.98 (0.92-1.03), ( p=0.42 )</td>
<td></td>
</tr>
<tr>
<td><strong>Health Outcomes at 3 years of age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotinine</td>
<td>1.03 (0.95-1.11), ( p=0.41 )</td>
<td>0.96 (0.89-1.02), ( p=0.18 )</td>
<td>0.99 (0.92-1.06), ( p=0.72 )</td>
</tr>
<tr>
<td><em>Trans</em>-3'-hydroxycotinine</td>
<td>1.04 (0.97-1.12), ( p=0.25 )</td>
<td>0.93 (0.87-0.99), ( p=0.03 )</td>
<td>0.99 (0.93-1.06), ( p=0.86 )</td>
</tr>
<tr>
<td>Mother quit smoking prior to pregnancy vs. Never Smoked</td>
<td>1.08 (0.66-1.71), ( p=0.74 )</td>
<td>1.00 (0.69-1.43), ( p=0.99 )</td>
<td>1.36 (0.92-1.99), ( p=0.11 )</td>
</tr>
<tr>
<td>Mother smoked into pregnancy vs. Never Smoked</td>
<td>1.38 (0.59-2.80), ( p=0.42 )</td>
<td>0.40 (0.14-0.92), ( p=0.05 )</td>
<td>0.94 (0.41-1.90), ( p=0.88 )</td>
</tr>
<tr>
<td>Any household smoking in early life vs. None</td>
<td>1.00 (0.54-1.73), ( p=1.0 )</td>
<td>0.85 (0.52-1.35), ( p=0.52 )</td>
<td>0.99 (0.59-1.59), ( p=0.97 )</td>
</tr>
<tr>
<td>Days mom was exposed in past two weeks of pregnancy</td>
<td>1.02 (0.96, 1.07), ( p=0.49 )</td>
<td>0.93 (0.89-0.99), ( p=0.03 )</td>
<td>0.97 (0.91-1.03), ( p=0.35 )</td>
</tr>
<tr>
<td><strong>Health Outcomes at 5 years of age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotinine</td>
<td>1.10 (1.01-1.18), ( p=0.02 )</td>
<td>0.96 (0.90-1.02), ( p=0.16 )</td>
<td>1.03 (0.97-1.10), ( p=0.27 )</td>
</tr>
<tr>
<td><em>Trans</em>-3'-hydroxycotinine</td>
<td>1.09 (1.01-1.17), ( p=0.02 )</td>
<td>0.95 (0.90-1.01), ( p=0.09 )</td>
<td>1.05 (0.99-1.11), ( p=0.10 )</td>
</tr>
</tbody>
</table>
### Health Outcomes at 1 year of age

<table>
<thead>
<tr>
<th>Health Outcomes at 1 year of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotinine</td>
</tr>
<tr>
<td>1.10 (0.99-1.21), p=0.06</td>
</tr>
<tr>
<td>0.94 (0.86-1.03), p=0.21</td>
</tr>
</tbody>
</table>

| Trans-3’-hydroxycotinine         |
| 1.04 (0.93-1.15), p=0.44        |
| 0.95 (0.87-1.04), p=0.27        |

### Health Outcomes at 3 years of age

<table>
<thead>
<tr>
<th>Health Outcomes at 3 years of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotinine</td>
</tr>
<tr>
<td>1.07 (0.96-1.17), p=0.19</td>
</tr>
<tr>
<td>0.99 (0.91-1.07), p=0.75</td>
</tr>
<tr>
<td>0.99 (0.92-1.06), p=0.72</td>
</tr>
</tbody>
</table>

| Trans-3’-hydroxycotinine         |
| 1.04 (0.94-1.14), p=0.46        |
| 0.97 (0.89-1.05), p=0.42        |
| 0.99 (0.93-1.06), p=0.86        |

### Health Outcomes at 5 years of age

<table>
<thead>
<tr>
<th>Health Outcomes at 5 years of age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cotinine</td>
</tr>
<tr>
<td>1.06 (0.94-1.18), p=0.32</td>
</tr>
<tr>
<td>0.99 (0.92-1.07), p=0.84</td>
</tr>
<tr>
<td>1.03 (0.97-1.10), p=0.27</td>
</tr>
</tbody>
</table>

| Trans-3’-hydroxycotinine         |
| 1.11 (1.00-1.22), p=0.04        |
| 0.98 (0.90-1.05), p=0.51        |
| 1.05 (0.99-1.11), p=0.10        |

Urinary concentrations were log-transformed and treated as a continuous variable in these bivariate logistic regression models against each health outcome. Results could not be generated for asthma at 1 year of age due to a low number of cases (n=7). Odds ratios (OR) and 95% confidence intervals (95% CI) from logistic regression models are reported. P-values less than or equal to 0.05 are bolded. OR are expressed per 1-unit increase in log-transformed (base 2) concentration of cotinine and 3HC.
Table 3.5. Cotinine and trans-3'-hydroxycotinine as a categorical predictor of childhood asthma & intermediates at 1, 3 and 5 years of age

<table>
<thead>
<tr>
<th></th>
<th>Recurrent Wheeze OR (95% CI), p-value</th>
<th>Atopy OR (95% CI), p-value</th>
<th>Asthma Diagnosis OR (95% CI), p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Health Outcomes at 1 year of age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotinine</td>
<td>1.75 (1.16-2.61), p=0.01</td>
<td>0.56 (0.37-0.84), p=0.01</td>
<td>-</td>
</tr>
<tr>
<td>Trans-3'-hydroxycotinine</td>
<td>1.44 (0.93-2.16), p=0.09</td>
<td>0.60 (0.39-0.89), p=0.02</td>
<td>-</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Health Outcomes at 3 years of age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotinine</td>
<td>1.34 (0.87-2.01), p=0.17</td>
<td>0.79 (0.54-1.12), p=0.19</td>
<td>0.96 (0.64-1.39), p=0.82</td>
</tr>
<tr>
<td>Trans-3'-hydroxycotinine</td>
<td>1.25 (0.81-1.88), p=0.31</td>
<td>0.60 (0.40-0.87), p=0.01</td>
<td>0.98 (0.66-1.42), p=0.92</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Health Outcomes at 5 years of age</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cotinine</td>
<td>1.44 (0.90-2.25), p=0.12</td>
<td>0.77 (0.55-1.06), p=0.12</td>
<td>1.15 (0.82-1.60), p=0.40</td>
</tr>
<tr>
<td>Trans-3'-hydroxycotinine</td>
<td>1.24 (0.76-1.97), p=0.37</td>
<td>0.81 (0.58-1.12), p=0.21</td>
<td>1.15 (0.82-1.60), p=0.42</td>
</tr>
</tbody>
</table>

Urinary concentrations were log-transformed and treated as a categorical variable, with those considered to have a concentration indicative of secondhand smoke (SHS) exposure compared to those with lower concentrations. Results could not be generated for asthma at 1 year of age due to a low number of cases (n=7). Odds ratios (OR) and 95% confidence intervals (95% CI) from logistic regression models are reported. OR are expressed per comparing higher (>=0.25 ng/ml) with lower cotinine (<0.25 ng/ml) and higher (>=0.50 ng/ml) with lower 3HC (<0.50 ng/ml) concentration. P-values less than or equal to 0.05 are bolded. The range of the log-transformed cotinine concentration was from 10.47 to 8.41, while the range of log-transformed 3HC concentrations was from -8.11 to 11.21.
Table 3.6. Multiplicative change in cotinine and trans-3’-hydroxycotinine by breastfeeding status, adjusted for maternal or household smoking exposure

<table>
<thead>
<tr>
<th>Breastfeeding Status</th>
<th>Multiplicative change in urinary cotinine concentration (ng/mL)</th>
<th>Multiplicative change in urinary trans-3’-hydroxycotinine concentration (ng/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Unadjusted</td>
<td>Adjusted for Maternal Smoking during Pregnancy</td>
</tr>
<tr>
<td>Partial vs. Exclusive</td>
<td>1.29 (1.07-1.55), <strong>0.01</strong></td>
<td>1.07 (0.90-1.28), 0.44</td>
</tr>
<tr>
<td>None vs. Exclusive</td>
<td>1.14 (0.96-1.35), 0.14</td>
<td>1.05 (0.89-1.24), 0.55</td>
</tr>
</tbody>
</table>

Linear regression models were used to model each maternal diet variable against each urinary biomarker. Urinary concentrations were log-transformed before fitting regression models. Model coefficients were exponentiated to report the multiplicative change in untransformed metabolite concentrations, their 95% confidence intervals, and p-value. P-values less than or equal to 0.05 are bolded. A multiplicative change of 1.27, for example, corresponds with a 29% increase in the urinary metabolite concentration compared to the reference group. In the model that adjusted for maternal smoking, those who had a mother report actively smoking during pregnancy (n=22) had 24.9 times (95% CI 15.37-39.43) higher cotinine concentrations and a 35.74 times (95% CI 22.22-57.48) higher 3HC concentration than those who did not. In the model that adjusted for the occurrence of household smoking in early life, those who lived in a home where household smoking had occurred had a 4.78 times (95% CI 3.71-6.16) higher cotinine concentrations and a 7.42 times (95% CI 5.73-9.59) higher 3HC concentration than those who had not has smoking occur at the home.
Table 3.7. Urinary Cotinine Levels in Infants by Household Tobacco Smoke Exposure and Breastfeeding Status

<table>
<thead>
<tr>
<th></th>
<th>Cotinine Geometric Mean (95% CI), ng/mL</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Exclusively Breastfed (n=890)</td>
<td>Partially Breastfed (n=369)</td>
</tr>
<tr>
<td>Household Smoke Exposure (n=163, 11%)</td>
<td>0.40 (0.26-0.64)</td>
<td>0.56 (0.26-1.20)</td>
</tr>
<tr>
<td>No Household Exposure (n=1269, 89%)</td>
<td>0.09 (0.09-0.10)</td>
<td>0.09 (0.08-0.10)</td>
</tr>
<tr>
<td>Mother is non-smoker (n=1410, 98%)</td>
<td>0.10 (0.09-0.11)</td>
<td>0.10 (0.09-0.12)</td>
</tr>
<tr>
<td>Household Smoke Exposure (n=143, 10%)</td>
<td>0.27 (0.19-0.39)</td>
<td>0.33 (0.17-0.64)</td>
</tr>
<tr>
<td>No Household Exposure (n=1267, 90%)</td>
<td>0.09 (0.09-0.10)</td>
<td>0.09 (0.08-0.10)</td>
</tr>
<tr>
<td>Mother is a smoker (n=22, 2%)</td>
<td>74.65 (43.21-128.96)</td>
<td>33.34 (5.48-202.93)</td>
</tr>
</tbody>
</table>

Geometric mean concentrations of cotinine are reported based on whether the subject was classified as having a smoker living at the home, a mother who smoked during pregnancy, and whether and how exclusively they were breastfed in the first few months of life. ANOVA tests compared whether the mean log-transformed urinary cotinine concentrations differed significantly based on breastfeeding status for each of the conditions held on the left-hand column. When these ANOVA tests were repeated for 3HC concentrations, the only significant p-value was seen when the mother was an active smoker (p<0.001) (data not shown).

**Breastfeeding**

The presence of a smoker at the home resulted in the greatest average increase in urinary cotinine concentrations amongst those partially breastfed (+6.30 ng/mL), followed by never breastfed (+4.47 ng/mL) then those exclusively breastfed (+4.28 ng/mL) when compared to those who did not have a smoker at the home. Those who never had breastfed their child had the highest proportion of reported household smokers (19.7%), when compared to those who partially (11.7%) or exclusively breastfed their child in early life (9.7%). Those who were not breastfed in early life had significantly higher concentration of cotinine. However, once adjusted for even a single predictor relating to second-hand smoke exposure, breastfeeding difference was no longer statistically significant (Table 3.6). Breastfeeding appears to increase an infant’s nicotine exposure only when the mother is a smoker (Table 3.7).
Figure 3.1. Box plots of urinary cotinine concentrations by breastfeeding status and maternal smoking status.

Boxplots show the median and interquartile range, while whisker lines reach to the minimum and maximum log-transformed cotinine concentration for each categorization of maternal smoking in pregnancy by breastfeeding status.
Figure 3.2. Violin plots of urinary cotinine concentrations by breastfeeding status and presence of a household smoker.

Violin curves overlay boxplots to describe in more detail the distribution of log-transformed cotinine concentration. Boxplots show the median and interquartile range, while whisker lines reach to the minimum and maximum values of log-transformed cotinine concentration for each categorization of maternal smoking in pregnancy by breastfeeding status.

Infant and Maternal Diet

When included in multivariable linear regression models, this composite measure of a prudent diet was significantly associated with urinary concentrations of cotinine ($\beta = -0.12$, $p = 0.04$), but not 3HC ($\beta = 0.03$, $p = 0.64$). A likelihood ratio test (LRT) found that the addition of the composite prudent diet score variable did slightly improve the fit of the cotinine model, with the $R^2$ increasing from 29.9% to 30.1% ($p = 0.04$). The addition of this variable to the 3HC model did not improve its performance and did not increase the $R^2$ (from 38.8% to 40.3% ($p = 0.64$). It may have only slightly improved the cotinine prediction model because it had fewer predictors than the 3HC model. Bivariate analysis of a prudent/healthy maternal diet found a negative relationship with the infant’s urinary cotinine ($\beta = -0.22$, $p = 0.001$) and 3HC concentrations ($\beta = -0.18$, $p = 0.01$).
When the more-specific measure of average daily vegetable intake during pregnancy was added to multivariable prediction models did not help predict cotinine ($\beta=0.01$, $p=0.66$) or 3HC concentrations ($\beta=0.02$, $p=0.53$). A likelihood ratio test (LRT) found that the addition of the average daily vegetable intake variable did not improve the fit of the cotinine model, with the $R^2$ unchanged. The addition of this variable to the 3HC model did not improve the fit. Maternal vegetable intake was not associated with urinary biomarker concentrations on its own and did not change when used in the subset of subjects who were exclusively breastfed and lacked a household smoker ($n=804$) were examined. In infants exclusively breastfed, incorporating the average daily vegetable intake score variable did not improve the fit of the cotinine model (LRT $p=0.69$). Similar results occurred when adding the prudent/healthy diet score to models in a subset of those who were exclusively breastfed ($n=890$).

More specific consideration of dietary sources of nicotine using a combination of food frequency data, Health Canada portion guidelines, and existing literature of the nicotine content in particular foods (see Appendix B) found a small association between maternal dietary nicotine intake and their infant’s urinary concentrations of cotinine (Multiplicative change of 0.99, $p=0.04$) but not 3HC (Multiplicative change of 0.99, $p=0.15$). In a subset of those with a low urinary concentration of cotinine (<0.25ng/mL), exclusively breastfed with no maternal smoking or household smoking exposure ($n=555$), the association reversed to slightly positive but without statistical significance (Multiplicative change of 1.000001, $p=0.85$) (see Appendix B).
Table 3.8. Multiplicative change in urinary cotinine and *trans*-3′-hydroxycotinine concentration by maternal diet

<table>
<thead>
<tr>
<th>Multiplicative change in urinary cotinine concentration (ng/mL)</th>
<th>Prudent/Healthy Diet Score</th>
<th>Exclusively Breastfed (n=890)</th>
<th>Partially Breastfed (n=369)</th>
<th>Never Breastfed (n=173)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prudent/Healthy Diet Score</td>
<td>0.86 (0.79-0.94), p=0.001</td>
<td>0.88 (0.79-0.97), p=0.01</td>
<td>0.85 (0.70-1.03), p=0.09</td>
<td>0.90 (0.69-1.19), p=0.48</td>
</tr>
<tr>
<td>Average daily vegetable intake</td>
<td>0.97 (0.92-1.02), p=0.22</td>
<td>0.99 (0.93-1.06), p=0.85</td>
<td>0.92 (0.83-1.03), p=0.16</td>
<td>0.98 (0.85-1.12), p=0.73</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Multiplicative change in urinary <em>trans</em>-3′-hydroxycotinine concentration (ng/mL)</th>
<th>Prudent/Healthy Diet Score</th>
<th>Exclusively Breastfed (n=890)</th>
<th>Partially Breastfed (n=369)</th>
<th>Never Breastfed (n=173)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prudent/Healthy Diet Score</td>
<td>0.88 (0.81-0.97), p=0.01</td>
<td>0.93 (0.83-1.04), p=0.19</td>
<td>0.84 (0.69-1.03), p=0.10</td>
<td>0.81 (0.61-1.09), p=0.17</td>
</tr>
<tr>
<td>Average daily vegetable intake</td>
<td>0.95 (0.90-1.00), p=0.06</td>
<td>0.95 (0.89-1.02), p=0.16</td>
<td>0.97 (0.87-1.08), p=0.59</td>
<td>0.94 (0.81-1.09), p=0.40</td>
</tr>
</tbody>
</table>

Linear regression models were used to model each maternal diet variable against each urinary biomarker. Urinary concentrations were log-transformed before fitting regression models. Model coefficients were exponentiated to report the multiplicative change in untransformed metabolite concentrations, their 95% confidence intervals, and p-value. P-values less than or equal to 0.05 are bolded.
Figure 3.3. Scatterplots of maternal prudent/healthy diet scores and infant’s urinary cotinine concentration (top) and trans-3′-hydroxycotinine concentration (bottom)
3.5. Discussion

Findings from this study allow us to make interpretations about general smoking prevalence and behaviours, the risk of childhood asthmatic disease as a result of tobacco smoke exposure, and whether a breastfeeding mother's dietary sources of nicotine are relevant to the exposure of their breastfed children to nicotine. It appears that roughly one-fifth of the infants in our sample could be categorized as having some light SHS exposure in their early life. Higher cotinine and 3HC concentrations are associated with a slight increase in the risk of recurrent wheeze in childhood, but not allergy or asthma. Breastfeeding only increased an infant’s urinary metabolite concentrations when the mother had been or was a smoker, and a maternal diet high in healthy vegetables, or vegetables containing nicotine was not related to concentrations detected in breastfed infants with no household or maternal smoking exposure.

Smoking behaviours

Overall, the findings of this cohort offer encouraging insight into the smoking behaviours of mothers and families. A pregnancy cohort from Quebec, Canada found that 13-25% of pregnant women will continued to smoke during pregnancy in a sample collected between 1998 and 2009 (Bérard, Zhao, & Sheehy, 2016), but only 2-3% of our sample smoked throughout their pregnancy. Interestingly, 3% reported smoking going into pregnancy and not quitting during the pregnancy, but only 2% reported actively smoking during the prenatal questionnaire. Overall smoking rates during pregnancy have been dropping over time, with a 2006 estimated national average of 10.5%, and notable provincial variation and highest smoking rates found in the Northern Territories (Al-Sahab, Saqib, Hauser, & Tamim, 2010). A 2010 population-based US study found that approximately 25% of women smoke before pregnancy, 12% during pregnancy, and 17% after delivery (Tong et al., 2013). By comparison, 23.7% of mothers in our study smoked at some point before their pregnancy, 18.7% had already quit prior to their pregnancy, 5% smoked at the start of their pregnancy, and only 3% never quit throughout their pregnancy.

Of those who did report smoking to occur at the home by inhabitants or visitors, the majority of smoking occurred outside (Table 3.2). This may be with intent to minimize exposure to the child and mother. Although most household smoking occurred outdoors, 22-23% of the infants in our sample could be categorized as having some level of
second-hand tobacco smoke exposure. More work can be done to increase awareness of SHS, particularly amongst those who rent or are in multi-family housing and are more vulnerable to exposure resulting from those smoking outside of their household.

While the majority of those in our study who were not actively smoking during pregnancy did not have a smoker living at the home in early life, those who did have a smoker at the home had higher concentrations of nicotine metabolites detected in their infant. Increasing public awareness that even “unseen” light second-hand and thirdhand smoke is detectable in infants and may impart some risk to the infant can help in furthering tobacco control and smoking cessation initiatives.

**Asthma risk**

Cotinine and 3HC alone in unadjusted logistic regression models were not strong predictors of childhood allergic and asthmatic disease, as would be expected for a biomarker of tobacco smoke exposure. Our findings suggest an increased odds of recurrent wheeze at 1 and 5 years of age, and reduced risk of atopy at 1 year of age with increased urinary concentrations. However, the lack of consistent findings at 3 years of age and a low number of cases are reason to be cautious in the interpretation of these results. Others have found urinary cotinine to predict the risk of recurrent wheezing but not asthma in early childhood, but that the directionality of effect estimates were unstable over time (Carlsten et al., 2012). The findings with reduced atopy risk adds to the inconsistencies seen in existing literature around passive smoking exposure and allergic sensitization (Ciaccio & Gentile, 2013; Thacher et al., 2016), and may be implicated by the immunosuppressive or dysregulating properties of nicotine (Piao et al., 2009). Odds ratios from the use of questionnaire responses to predict risk followed similar trends, with some significant increase in the risk of recurrent wheeze at 1 year of age, and some protection of atopy at 3 years of age.

Low and/or intermittent tobacco smoke exposure is not a clinically significant risk factor for asthmatic disease in childhood in our sample. Inconsistencies in effect sizes may be due a lack of power from a low number of cases. It is reasonable that we are not seeing a relationship between detected concentrations and clinical diagnosis because of implications with dietary routes of exposure. If the source of the nicotine exposure for these infants is predominantly or exclusively through vegetables, a source of anti-inflammatory and antioxidant agents, then;
A) The inflammatory properties of nicotine (and other chemical toxicants released by harmful tobacco smoke) may be counteracted by antioxidant nutrients and other protective properties of breastfeeding and/or a diet high in anti-inflammatory and antioxidant fruits and vegetables. This oxidative-stress relationship in childhood asthma and allergic disease risk has been described by others (Sordillo et al., 2019). Micronutrients delivered from a diet high in fruits, vegetables and antioxidant-containing foods can increase resiliency against oxidative stresses such as tobacco smoke (Wilson, Finkelstein, Blumkin, Best, & Klein, 2011).

B) If the nicotine is delivered to the infant through breast milk from mothers who eat vegetables known to contain small amounts of nicotine instead of being delivered by tobacco smoke, the metabolites of this nicotine cease to reflect risks associated with tobacco smoke and instead reflect the properties of a vegetable-rich diet (Hosseini, Berthon, Wark, & Wood, 2017). As a result, the concentrations may indicate protective exposures associated with a reduced asthma and allergy risk rather than an increased risk associated with tobacco smoke exposure.

Breastfeeding

Results corroborate that breast milk is an additional route of nicotine exposure for breastfed infants (Becker et al., 1999). One study found infant urinary cotinine levels to be 5 times higher in children of smoking mothers who were breastfed compared to children with smoking mothers who were not breastfed (Becker et al., 1999). When the mother is a non-smoker or there is no reported household smoker, there is a negligible difference in infant’s urinary cotinine concentration based on breastfeeding status. When the mother is a smoker, there is a significant difference in the infant’s urinary cotinine concentration based on breastfeeding status. Smokers who breastfeed should be encouraged to reduce or quit smoking and increase their intake of antioxidants rather than reduce or quit breastfeeding and maintain their smoking behaviour.

Our study also identified that those who breastfeed more also report less household smoking exposure. Of those who exclusively breastfeed, those who report
some household smoke exposure in early life have infants with a higher urinary cotinine and 3HC concentration than those without household exposure. Even with most household smoking reportedly occurring outdoors, the report of a smoker living at the home impacts how much tobacco smoke the infant is exposed to. This exposure likely occurs through light second-hand smoke, such as the infiltration of outdoor smoking through a window, or third-hand smoke from clothing and other fabrics that act as reservoirs from earlier smoke exposure.

**Infant and maternal diet**

The majority of the sample were exclusively breastfeeding at 3 months of age (62%), followed by those combining breastfeeding with formula (24%), and those strictly formula feeding (11%). Dietary sources of nicotine may be relevant when exploring exposure sources for a population with a notably low prevalence of tobacco smoking. A high-vegetable diet, which likely carries some detectable nicotine exposure, did not increase the urinary concentrations of any infants regardless of breastfeeding status. Daily average vegetable intake, and the healthy diet variable both were negatively associated with infants’ urinary concentrations of cotinine and *trans*-3’-hydroxycotinine. This finding is likely because these variables act as a proxy for other protective lifestyle factors. For example, those who had mothers that ate a healthier diet with more vegetables had a partner with higher education, and a higher household income, and less tobacco smoke exposure. Altogether, a higher vegetable diet may be indicative of higher socioeconomic status, a factor inherently linked to the use of cigarettes (Cui, Shooshtari, Forget, Clara, & Cheung, 2014). We found no association between maternal dietary nicotine intake and their infant’s urinary concentrations of cotinine and 3HC, even when restricting analysis to a group of infants where dietary nicotine sources becomes most possible. However, it remains reasonable that cotinine derived from a vegetable-rich diet may be protective of childhood asthma and wheeze, warranting more focused data collection and analysis (Hosseini et al., 2017).
3.6. Limitations and Future Directions

There are important limitations to this study. While the low prevalence of tobacco smoke exposure in this cohort should be celebrated, this cohort does slightly underestimate true exposure experienced by the Canadian population. The CHILD cohort is known to contain subjects with parents who are more affluent, educated, and allergic or predisposed to sensitivity than the general Canadian population. Mothers who smoke during pregnancy generally are of low socioeconomic status, non-immigrant, single, have a chronic disease and are not in attendance of prenatal classes nor linked to a family doctor (Al-Sahab et al., 2010; Cui et al., 2014). We acknowledge that this study population of urbanized, relatively affluent people is not reflective of the entire Canadian population, and that the findings of this study are not generalizable to all provinces.

There were higher proportions of subjects from the Manitoba-based and Vancouver sites that were available for this analysis when compared to the overall CHILD cohort which may impact results. There are also regional differences in smoking rates, with the Northern territories having the highest provincial rates of smoking during pregnancy (Al-Sahab et al., 2010). Specifically, those of lower income, rural homestead and those belonging to a visible minority where smoking prevalence is hypothesized to be higher are underrepresented and may therefore underrepresent the proportion of smoking exposure to expectant and new mothers in the Canadian population. Cotinine may still be a useful validation measure of smoking exposure in these populations of the Canadian public, as well as other jurisdictions where smoking prevalence is higher.

At the time of data collection for questionnaires used by this study, e-cigarette use was not yet popularized and marijuana use was still illegal in Canada. We recognize that we have been unable to assess for novel, non-cigarette tools for tobacco exposure, and encourage that future analysis make use of data on marijuana and e-cigarette use when characterizing tobacco smoke exposure.

Finally, the dietary data used for this project were not ideal for addressing our specific question of dietary sources of nicotine. The food frequency questionnaire did not separate nightshade-family vegetables (high in nicotine) from other vegetables (low in, or void of nicotine). Thus, our analysis was limited to variables representing exposure to all vegetables, of which an unknown portion are expected to be from the nightshade family. The amount is expected to vary by region, season, and cultural differences in food
preference. The potential for nightshade vegetables to contribute dietary nicotine to children needs further evaluations with modern levels of detection to better understand cotinine and 3HC concentrations as indicators of risk. As well, the food frequency questionnaire was completed during pregnancy so we are unable to determine how maternal diets change over time, and how this may impact their children. Future work should examine multiple exposure routes including food sources and dermal exposure to nicotine to enable better interpretation of lower concentrations of nicotine metabolites and any effect they may have on the risk of asthma or other inflammatory diseases in their children.

3.7. Conclusions

The majority of our sample had urinary concentrations indicative of very light second-hand smoke and thirdhand smoke exposure, and this exposure was reported not to occur as a result of the mother. Increasing cotinine and trans-3'-hydroxycotinine concentrations in infant’s were associated with a slightly increased odds of having recurrent wheeze at 1 and 5 years of age, and a reduced odds of atopy at 1 year of age. These relationships were not as strong in a subsample of those exclusively breastfed, which may indicate that breast milk inhibits or counters the inflammatory effect of nicotine. Breastfeeding is an important route of nicotine exposure for second-hand and thirdhand smoke exposure and can increase an infant’s exposure when the mother is a smoker. Breastfeeding does not appear to increase a child’s exposure to nicotine when in a household where other smoking occurs, perhaps because the majority of household smoking occurs outdoors. Our findings do not suggest that a maternal diet high in vegetables or nicotine-containing vegetables can result in an increased intake of nicotine to the child, even when considering exclusively breastfed infants where dietary sources are most relevant. However, this analysis is limited to the use of data that does not offer the level of specificity needed to determine how a vegetable-rich diet influences exposure and any risk as a result of dietary nicotine versus tobacco-smoke derived nicotine. More specific analysis is needed to confirm these findings, using food-specific micronutrient and nicotine testing and tracking of nicotine concentrations from food source to infant.
3.8. References


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Chapter 4.

Conclusions

4.1. Overall Contributions

This thesis has been able to address the 3 overall objectives of the project. It implements quantitative analysis on questionnaires, biomarker and clinical assessment data from the CHILD cohort study, and regression modelling approaches that combine traditional and machine learning conventions for variable selection.

What are the levels and key sources of nicotine metabolites in a population of Canadian infants?

The geometric mean cotinine concentration of the infants was 0.12ng/mL (95% CI: 0.11-0.13ng/mL), while the geometric mean 3HC concentration was 0.22 ng/mL (95%CI: 0.21-0.24ng/mL). While 76% and 89% of the sample had detectable concentrations of cotinine and 3HC, the majority (77%) of infants have concentrations indicative of light SHS, THS, or none at all. Second-hand smoke exposure during pregnancy, the amount of smoking that occurs at the home during pregnancy and after the child’s birth, and the smoking status of the mother were key predictors of variation in urinary metabolite concentrations of infants at 3-4 months of age. Factors relating to socioeconomic status, housing, breastfeeding, and third-hand smoke exposure were also important to explaining concentrations detected in the infants.

Does diet contribute to low but detectable levels of cotinine in children?

Breastfeeding was an important predictor of nicotine metabolite concentrations in infants, because it serves as an additional route of exposure to nicotine. While it is possible that some of the nicotine passed from mother to child through breastmilk could come from dietary sources (ie. Nightshade vegetables), our findings indicate that breastfeeding only made a difference to an infant’s measurable exposure in settings where the mother had reported smoked. A maternal diet high in vegetables was not associated with a child’s urinary metabolite concentrations when controlling for tobacco smoke exposure.
What are the clinical implications and relevance of these concentrations?

There was no significant relationship between concentrations detected in these infants and asthma diagnosis in childhood. However, there is a relationship with recurrent wheeze at 1 and 5 years of age, but not at 3 years. This inconsistency over time warrants caution and more research is needed on subclinical manifestations of inflammation that may be linked to light SHS, THS exposure, and dietary nicotine consumption.

4.2. Discussion

While our findings indicate that maternal smoking rates are very low, pregnant women and new mothers may still be exposed to smoking by others at their home. Pregnant women, mothers and households expecting a child smoke less than the national average, with those who are younger, less educated, and of lower socioeconomic status smoking the most (Cui, Shooshtari, Forget, Clara, & Cheung, 2014). About half of all women that smoke daily before their pregnancy will continue to smoke during their pregnancy, though the majority of this will be ‘light’ smoking (Lange, Probst, Rehm, & Popova, 2018), which is defined as 10-14 or fewer cigarettes per day (Cui et al., 2014; Lange et al., 2018). This definition is problematic, as it labels a considerable consumption of cigarettes in a way that can be perceived as carrying very little risk. Family dynamics, housing, and physiological changes in metabolism are all important factors when encouraging pregnant women or those who may become pregnant to quit smoking, as well as other members of their household. Globally, Canada has some of the highest proportions of smoking during pregnancy, though this is driven primarily by the higher smoking rates during pregnancy seen in the Northern Territories (Al-Sahab, Saqib, Hauser, & Tamim, 2010; Lange et al., 2018). To facilitate the success of smoking cessation, having other household members on-board with quitting by engaging in smoking cessation or consciously reducing their smoking is key. One study exploring the paternal narrative of smoking cessation found that those who were aware of the dangers of passive smoke were more likely to quit, and that parenthood did inspire fathers to modify their smoking behaviours (Bottorff, Radsma, Kelly, & Oliffe, 2009). For knowledge translation efforts to be effective, more research is needed on the health impacts of low but detectable levels of smoke exposure, as well as the reach and persistence of third-hand smoke in the home. Only in the past few years have researchers begun to tease
out the health effects and pervasiveness of third-hand smoke (Hang et al., 2017; Leung, Ho, Wang, & Lam, 2018; Northrup, Jacob, et al., 2016; Northrup, Matt, Hovell, Khan, & Stotts, 2016). As a relatively new phenomena in public health, the public at large lacks awareness and understanding of third-hand smoke, making THS an indispensable component of tobacco control (Diez-Izquierdo et al., 2018).

Our findings indicate that increased exposure to tobacco smoke, albeit light, may be associated with an increased risk of recurrent wheeze. While not enough for a clinical diagnosis of asthma, recurrent wheezing in early life is a concern for the future development of asthma and an increased likelihood of other comorbidities such as allergy.

Exploration of the data found that breastfeeding is an additional route of nicotine exposure for infants, but that any difference in nicotine consumption as a result of breastfeeding is only significant when the in context of having a mother who smokes or smoked during pregnancy. Infants who were breastfed more had lower concentrations of cotinine and 3HC, and less reported exposure to SHS. The majority of the infants in our study were exclusively being breastfed and may be more influenced by the mother’s diet than those who weren’t. While we couldn’t confirm whether a diet rich in nicotine-containing vegetables contributed small but detectable concentrations of nicotine to a breastfed child with the data available, this concept should not be dismissed. Any nicotine sourced from a vegetable-rich diet would be a marker of a diet high in anti-inflammatory and antioxidizing properties, which could abate any risk of inflammatory disease associated with light exposure to second-hand and third-hand smoke (Litonjua et al., 2006; Martindale et al., 2005; Sordillo et al., 2019). The potential for oxidative balancing in early life is of particular importance in populations with little smoking exposure and relatively high prevalence of breastfeeding (Hovell, Zakarian, Wahlgren, Matt, & Emmons, 2000a; Sordillo et al., 2019).

4.3. General Conclusions and Future Outlook

In the context of reduced tobacco smoking in urbanized centers, particularly in affluent populations, the use of biomarkers to accurately depict an infant’s true exposure to second-hand and third-hand smoke has become problematic. Researchers need to combine qualitative and quantitative data on smoking behaviours to better educate the
public and prevent exposure to second-hand and third-hand smoke in infants and young children. We have added to a growing body of research stating that a combination of biomarkers and questionnaires should be used to assess tobacco smoke exposure, with particular emphasis on household characteristics that may affect the persistence of tobacco smoke in indoor air (Hovell, Zakarian, Wahlgren, Matt, & Emmons, 2000b; Kalkbrenner et al., 2010).

Pregnancy presents an opportune time to promote smoking cessation or reduction, not only for the mothers but also for others in the home who may smoke. This project encourages a need to improve our ability to accurately measure tobacco smoke exposure in populations with light exposure, and to recognize the possible implications of non-smoking sources of nicotine. The sub-clinical implications of low but persistent nicotine exposure through THS or intermittent SHS require further investigation. The results from this thesis can inform decision-makers and provide recommendation of how biomarkers and questionnaires should be used as tools to assess exposure to tobacco smoke, and how the implication of THS should be further researched to better inform knowledge translation and tobacco control measures. The challenges of public policy will be more complicated when addressing environmental tobacco smoke exposure. In addition to physicians, parents, and teachers, stakeholders in real estate, bylaw enforcement, public and private transportation operators must be considered in order to make control of low-level smoke exposure as ubiquitous as the smoke itself (Burton, 2011; Jacob et al., 2017; Matt et al., 2011; Northrup, Jacob, et al., 2016). While the Canadian population overall is reducing the rates of smoking, particularly during pregnancy and when around children – both as a result of policy and social pressures, our understanding of cotinine as a measure of smoking and subsequent clinical risk needs to change.
4.4. References


Appendix A.

Supplemental Information for Chapter 2: Assessing tobacco smoke exposure in Canadian infants using questionnaires, biomarkers, and machine learning

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Of the 3,455 children in the CHILD Study, 2,607 had urine samples collected at the 3-4 month home visit. 2,607 urine samples are available, with 2,509 of these samples remaining after excluding those with inconclusive results errors. These results errors include results that were duplicates, experienced interference with the sample, had insufficient volume for testing, or with a specific gravity measures above a 3 standard deviation cutoff. Of the 2,509 participants with metabolite data, 589 (23.5%) had a cotinine concentration below the LOD, and 271 (10.8%) had a 3HC concentration below the LOD. A subset of these 2,509 who have complete data, including those who could not have a result reported because the concentration was below the level of detection (0.03ng/mL) were selected as the current sample size. This sample was then restricted to those who also had complete information for all demographic and potential predictors of tobacco smoke exposure, limiting the sample size to 2,017.
### Table A.1a. Distribution of Metabolite Concentrations by Smoking Exposure

<table>
<thead>
<tr>
<th>Predictor Variable</th>
<th>% (N)</th>
<th>Geometric mean urinary Cotinine (95% CI), ng/mL</th>
<th>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Prenatal maternal smoking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never Smoked</td>
<td>91.9% (n=1853)</td>
<td>0.10 (0.09-0.10)</td>
<td>0.18 (0.17-0.19)</td>
</tr>
<tr>
<td>Quit during pregnancy</td>
<td>4.4% (n=88)</td>
<td>0.82 (0.52-1.29)</td>
<td>2.30 (1.34-3.93)</td>
</tr>
<tr>
<td>Did not quit smoking</td>
<td>3.9% (n=76)</td>
<td>1.15 (0.62-2.16)</td>
<td>3.24 (1.68-6.26)</td>
</tr>
<tr>
<td><strong>Maternal smoking status in pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never Smoked</td>
<td>97.5% (n=1967)</td>
<td>0.10 (0.10-0.11)</td>
<td>0.20 (0.19-0.21)</td>
</tr>
<tr>
<td>Daily or Occasional Smoker</td>
<td>2.6% (n=50)</td>
<td>7.13 (4.18-12.14)</td>
<td>21.96 (12.21-39.48)</td>
</tr>
<tr>
<td><strong>Pre-prenatal maternal smoking</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never Smoked</td>
<td>76.1% (n=1535)</td>
<td>0.09 (0.09-0.10)</td>
<td>0.17 (0.16-0.18)</td>
</tr>
<tr>
<td>Quit prior to pregnancy</td>
<td>17.7% (n=358)</td>
<td>0.12 (0.10-0.15)</td>
<td>0.27 (0.23-0.31)</td>
</tr>
<tr>
<td>Did not quit prior to pregnancy</td>
<td>6.1% (n=124)</td>
<td>1.81 (1.19-2.77)</td>
<td>5.16 (3.19-8.34)</td>
</tr>
<tr>
<td><strong>Was the mother exposed to a smoker at the home during pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes, Regularly</td>
<td>1.2% (n=25)</td>
<td>2.04 (0.97-4.32)</td>
<td>6.26 (2.87-13.69)</td>
</tr>
<tr>
<td>Yes, Occasionally</td>
<td>1.7% (n=35)</td>
<td>0.85 (0.44-1.62)</td>
<td>2.39 (1.16-4.92)</td>
</tr>
<tr>
<td>No</td>
<td>97.0% (n=1957)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.22)</td>
</tr>
<tr>
<td><strong>Household smoking during pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>88.9% (n=1794)</td>
<td>0.10 (0.09-0.10)</td>
<td>0.18 (0.17-0.19)</td>
</tr>
<tr>
<td>Yes</td>
<td>11.1% (n=223)</td>
<td>0.56 (0.44-0.71)</td>
<td>1.44 (1.11-1.87)</td>
</tr>
<tr>
<td><strong>Where household smoking occurs during pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Inside</td>
<td>1.0% (n=20)</td>
<td>3.00 (1.46-6.15)</td>
<td>8.58 (3.68-19.99)</td>
</tr>
<tr>
<td>Near Open Window or in Garage</td>
<td>1.9% (n=38)</td>
<td>0.91 (0.54-1.55)</td>
<td>2.62 (1.39-4.94)</td>
</tr>
<tr>
<td>Outside</td>
<td>8.8% (n=178)</td>
<td>0.47 (0.36-0.61)</td>
<td>1.19 (0.89-1.59)</td>
</tr>
<tr>
<td>Predictor Variable</td>
<td>% (N)</td>
<td>Geometric mean urinary Cotinine (95% CI), ng/mL</td>
<td>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</td>
</tr>
<tr>
<td>-------------------</td>
<td>-------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td><strong>Mother reports exposure during pregnancy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Recent Exposure</td>
<td>21.1% (n=425)</td>
<td>0.29 (0.24-0.35)</td>
<td>0.66 (0.54-0.81)</td>
</tr>
<tr>
<td>No recent exposures</td>
<td>78.9% (n=1592)</td>
<td>0.09 (0.08-0.10)</td>
<td>0.29 (0.24-0.35)</td>
</tr>
<tr>
<td><strong>Mother reports average daily exposure to smoke during pregnancy (continuous)</strong></td>
<td></td>
<td>p &lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>None</td>
<td>79.0% (n=1593)</td>
<td>0.09 (0.08-0.10)</td>
<td>0.17 (0.16-0.26)</td>
</tr>
<tr>
<td>0.25 hr/day</td>
<td>14.8% (n=299)</td>
<td>0.21 (0.18-0.26)</td>
<td>0.46 (0.38-0.57)</td>
</tr>
<tr>
<td>0.5-1 hr/day</td>
<td>3.6% (n=72)</td>
<td>0.49 (0.27-0.89)</td>
<td>1.10 (0.57-2.13)</td>
</tr>
<tr>
<td>2-4hrs/day</td>
<td>1.4% (n=29)</td>
<td>0.45 (0.19-1.07)</td>
<td>1.18 (0.46-3.02)</td>
</tr>
<tr>
<td>5-24hrs/day</td>
<td>1.2% (n=24)</td>
<td>1.91 (0.72-5.09)</td>
<td>6.05 (2.16-16.92)</td>
</tr>
<tr>
<td><strong>Mothers days exposure to smoke during pregnancy in past 2 weeks (continuous)</strong></td>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>None</td>
<td>78.9% (n=1592)</td>
<td>0.09 (0.08-0.10)</td>
<td>0.17 (0.16-0.18)</td>
</tr>
<tr>
<td>1 day</td>
<td>5.0% (n=100)</td>
<td>0.12 (0.09-0.16)</td>
<td>0.26 (0.19-0.35)</td>
</tr>
<tr>
<td>2 days</td>
<td>4.1% (n=83)</td>
<td>0.17 (0.12-0.23)</td>
<td>0.39 (0.28-0.54)</td>
</tr>
<tr>
<td>3-4 days</td>
<td>4.0% (n=80)</td>
<td>0.22 (0.15-0.32)</td>
<td>0.45 (0.30-0.69)</td>
</tr>
<tr>
<td>5-6 days</td>
<td>1.2% (n=24)</td>
<td>0.27 (0.14-0.51)</td>
<td>0.50 (0.27-0.94)</td>
</tr>
<tr>
<td>7-8 day</td>
<td>1.3% (n=27)</td>
<td>0.26 (0.15-0.47)</td>
<td>0.45 (0.22-0.94)</td>
</tr>
<tr>
<td>9-12 days</td>
<td>1.3% (n=27)</td>
<td>0.38 (0.18-0.80)</td>
<td>1.08 (0.46-2.55)</td>
</tr>
<tr>
<td>14 days (every day)</td>
<td>4.2% (n=84)</td>
<td>1.84 (1.09-3.10)</td>
<td>5.09 (2.87-9.03)</td>
</tr>
<tr>
<td><strong>Household smoking since birth</strong></td>
<td></td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
</tr>
<tr>
<td>No</td>
<td>87.8% (n=1771)</td>
<td>0.10 (0.09-0.10)</td>
<td>0.17 (0.16-0.19)</td>
</tr>
<tr>
<td>Yes</td>
<td>12.2% (n=246)</td>
<td>0.50 (0.38-0.65)</td>
<td>1.36 (1.02-1.81)</td>
</tr>
<tr>
<td>Predictor Variable</td>
<td>% (N)</td>
<td>Geometric mean urinary Cotinine (95% CI), ng/mL</td>
<td>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</td>
</tr>
<tr>
<td>--------------------------------------------------------</td>
<td>------------------------</td>
<td>------------------------------------------------</td>
<td>-------------------------------------------------------</td>
</tr>
<tr>
<td>Where household smoking occurs during child’s early life</td>
<td>Inside v. None</td>
<td>Inside v. None p&lt;0.001</td>
<td>Inside v. None p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>Window v. None p&lt;0.001</td>
<td>Window v. None p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>Outside v. None p&lt;0.001</td>
<td>Outside v. None p&lt;0.001</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Inside</td>
<td>0.5% (n=10)</td>
<td>2.07 (0.79-5.44)</td>
</tr>
<tr>
<td></td>
<td>Window v. None</td>
<td>1.6% (n=32)</td>
<td>1.30 (0.63-2.71)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>Outside v. None</td>
<td>0.43 (0.33-0.57)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td>11.2% (n=225)</td>
<td>0.43 (0.33-0.57)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.001</td>
<td></td>
<td>11.6 (0.86-1.57)</td>
</tr>
<tr>
<td>Cigarettes smoked daily at the home during child’s early life</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>91.0% (n=1836)</td>
<td>0.10 (0.09-0.10)</td>
</tr>
<tr>
<td></td>
<td>1-5 cigarettes/day</td>
<td>5.6% (n=113)</td>
<td>0.42 (0.29-0.60)</td>
</tr>
<tr>
<td></td>
<td>6-10 cigarettes/day</td>
<td>1.7% (n=35)</td>
<td>1.27 (0.64-2.50)</td>
</tr>
<tr>
<td></td>
<td>10+ cigarettes/day</td>
<td>1.6% (n=33)</td>
<td>2.80 (1.29-6.08)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td></td>
<td>7.93 (3.37-18.63)</td>
</tr>
<tr>
<td>Parent reports smoking exposure to child in early life</td>
<td>p&lt;0.0001</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>92.6% (n=1868)</td>
<td>0.11 (0.10-0.12)</td>
</tr>
<tr>
<td></td>
<td>Some</td>
<td>7.4% (n=149)</td>
<td>0.30 (0.22-0.40)</td>
</tr>
<tr>
<td>Days baby was exposed to smoke during the past week, early life (continuous)</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td></td>
<td>None</td>
<td>95.6% (n=1928)</td>
<td>0.11 (0.10-0.12)</td>
</tr>
<tr>
<td></td>
<td>1 day</td>
<td>2.1% (n=43)</td>
<td>0.33 (0.20-0.54)</td>
</tr>
<tr>
<td></td>
<td>2 days</td>
<td>0.7% (n=14)</td>
<td>0.26 (0.10-0.71)</td>
</tr>
<tr>
<td></td>
<td>3-5 days</td>
<td>0.5% (n=9)</td>
<td>0.19 (0.06-0.62)</td>
</tr>
<tr>
<td></td>
<td>6-7 days</td>
<td>1.1% (n=23)</td>
<td>1.30 (0.46-3.64)</td>
</tr>
<tr>
<td></td>
<td>p&lt;0.0001</td>
<td></td>
<td>4.11 (1.55-10.91)</td>
</tr>
<tr>
<td>Predictor Variable</td>
<td>% (N)</td>
<td>Geometric mean urinary Cotinine (95% CI), ng/mL</td>
<td>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</td>
</tr>
<tr>
<td>--------------------</td>
<td>------------------------</td>
<td>--------------------------------------------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td>Hours of smoke exposure in the past week to child, early life</td>
<td>p&lt;0.0001</td>
<td>p&lt;0.0001</td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>95.6% (n=1929)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.22)</td>
</tr>
<tr>
<td>10 hours /week</td>
<td>3.7% (n=74)</td>
<td>0.37 (0.24-0.58)</td>
<td>1.02 (0.64-1.61)</td>
</tr>
<tr>
<td>10 to 20 hours/week</td>
<td>0.2% (n=5)</td>
<td>0.78 (0.10-6.34)</td>
<td>1.60 (0.14-18.76)</td>
</tr>
<tr>
<td>More than 20 hours/week</td>
<td>1.0% (n=20)</td>
<td>1.20 (0.29-5.04)</td>
<td>4.01 (1.01-15.85)</td>
</tr>
</tbody>
</table>

The proportion and crude number of sample subjects that corresponds to each level of household characteristic variables is reported to the nearest whole number. The geometric mean (95% Confidence interval) of the corrected and log-transformed Cotinine distribution for each level of each variable is also reported to the nearest two decimal places. P-values represent test for comparing means between levels of each predictor. P-values less than 0.05 are bolded.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>% (N)</th>
<th>Geometric mean urinary Cotinine (95% CI), ng/mL</th>
<th>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Dwelling Type</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Single Family</td>
<td>55.8% (n=1470)</td>
<td>0.10 (0.10-0.11)</td>
<td>0.20 (0.18-0.22)</td>
</tr>
<tr>
<td>Multi-Family or Apartment</td>
<td>25.8% (n=521)</td>
<td>0.15 (0.13-0.18)</td>
<td>0.29 (0.25-0.34)</td>
</tr>
<tr>
<td>Trailer or Other</td>
<td>1.2% (n=26)</td>
<td>0.24 (0.11-0.49)</td>
<td>0.53 (0.23-1.19)</td>
</tr>
<tr>
<td><strong>Carpeted Flooring</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carpeted Living Space (LS)</td>
<td>26.7% (n=539)</td>
<td>0.13 (0.11-0.15)</td>
<td>0.24 (0.20-0.27)</td>
</tr>
<tr>
<td>LS Not Carpeted</td>
<td>73.3% (n=1478)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.22 (0.20-0.24)</td>
</tr>
<tr>
<td>Carpeted Mom’s Room (MR)</td>
<td>50.0% (n=1009)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.23)</td>
</tr>
<tr>
<td>MR not carpeted</td>
<td>50.0% (n=1008)</td>
<td>0.12 (0.11-0.13)</td>
<td>0.24 (0.22-0.27)</td>
</tr>
<tr>
<td>Carpeted Child’s Room (CR)</td>
<td>39.9% (n=804)</td>
<td>0.10 (0.09-0.12)</td>
<td>0.20 (0.18-0.22)</td>
</tr>
<tr>
<td>CR Not Carpeted</td>
<td>42.5% (n=857)</td>
<td>0.11 (0.10-0.13)</td>
<td>0.21 (0.19-0.23)</td>
</tr>
<tr>
<td>*356 no child’s room</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Air Conditioning (AC)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>55.3% (n=1115)</td>
<td>0.12 (0.11-0.13)</td>
<td>0.21 (0.18-0.23)</td>
</tr>
<tr>
<td>Central AC</td>
<td>49.2% (n=992)</td>
<td>0.10 (0.09-0.11)</td>
<td>0.20 (0.18-0.22)</td>
</tr>
<tr>
<td>Window or Portable Unit</td>
<td>19.9% (n=402)</td>
<td>0.17 (0.14-0.20)</td>
<td>0.38 (0.31-0.46)</td>
</tr>
<tr>
<td><strong>Rent vs. Own Home</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rent</td>
<td>34.3% (n=692)</td>
<td>0.19 (0.16-0.22)</td>
<td>0.36 (0.31-0.43)</td>
</tr>
<tr>
<td>Own</td>
<td>90.1% (n=1817)</td>
<td>0.10 (0.09-0.11)</td>
<td>0.19 (0.18-0.21)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>% (N)</td>
<td>Geometric mean urinary Cotinine (95% CI), ng/mL</td>
<td>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</td>
</tr>
<tr>
<td>-----------------------------------</td>
<td>---------</td>
<td>-------------------------------------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td><strong>Bedrooms in Home</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No bedrooms</td>
<td>&lt;1% (n=2)</td>
<td>0.14 (N/A)</td>
<td>0.99 (N/A)</td>
</tr>
<tr>
<td>1-3 bedrooms</td>
<td>73.2% (n=1476)</td>
<td>0.12 (0.11-0.13)</td>
<td>0.24 (0.22-0.26)</td>
</tr>
<tr>
<td>4-6 bedrooms</td>
<td>26.3% (n=531)</td>
<td>0.10 (0.09-0.12)</td>
<td>0.19 (0.16-0.22)</td>
</tr>
<tr>
<td>7+ bedrooms</td>
<td>0.4% (n=8)</td>
<td>0.08 (0.03-0.22)</td>
<td>0.15 (0.05-0.48)</td>
</tr>
<tr>
<td><strong>Area Rug locations</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basement (B)</td>
<td>31.6% (n=637)</td>
<td>0.10 (0.09-0.11)</td>
<td>0.20 (0.17-0.22)</td>
</tr>
<tr>
<td>Not in Basement</td>
<td>37.5% (n=756)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.24)</td>
</tr>
<tr>
<td>No Basement</td>
<td>30.9% (n=624)</td>
<td>0.15 (0.13-0.17)</td>
<td>0.27 (0.24-0.31)</td>
</tr>
<tr>
<td>Kitchen (K)</td>
<td>18.2% (n=368)</td>
<td>0.12 (0.10-0.14)</td>
<td>0.21 (0.17-0.25)</td>
</tr>
<tr>
<td>Not in Kitchen</td>
<td>81.8% (n=1649)</td>
<td>0.12 (0.11-0.13)</td>
<td>0.23 (0.21-0.25)</td>
</tr>
<tr>
<td>Living Space (LS)</td>
<td>45.0% (n=907)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.20 (0.18-0.22)</td>
</tr>
<tr>
<td>Not in Living Space</td>
<td>55.0% (n=1110)</td>
<td>0.12 (0.11-0.13)</td>
<td>0.24 (0.22-0.27)</td>
</tr>
<tr>
<td>Mom’s bedroom (MB)</td>
<td>14.0% (n=282)</td>
<td>0.11 (0.10-0.13)</td>
<td>0.24 (0.20-0.29)</td>
</tr>
<tr>
<td>Not in Mom’s bedroom</td>
<td>86.0% (n=1735)</td>
<td>0.12 (0.11-0.13)</td>
<td>0.22 (0.20-0.24)</td>
</tr>
<tr>
<td>Bathroom (B)</td>
<td>35.1% (n=707)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.19 (0.17-0.22)</td>
</tr>
<tr>
<td>Not in Bathroom</td>
<td>64.9% (n=1310)</td>
<td>0.12 (0.11-0.13)</td>
<td>0.24 (0.22-0.27)</td>
</tr>
<tr>
<td><strong>Car Ownership</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Cars</td>
<td>6.0% (n=122)</td>
<td>0.20 (0.14-0.28)</td>
<td>0.45 (0.31-0.64)</td>
</tr>
<tr>
<td>1 Car</td>
<td>41.4% (n=835)</td>
<td>0.12 (0.10-0.13)</td>
<td>0.21 (0.19-0.24)</td>
</tr>
<tr>
<td>2 Car</td>
<td>46.7% (n=942)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.23)</td>
</tr>
<tr>
<td>3+ Cars</td>
<td>5.9% (n=118)</td>
<td>0.11 (0.08-0.15)</td>
<td>0.28 (0.20-0.39)</td>
</tr>
</tbody>
</table>

The proportion and crude number of sample subjects that corresponds to each level of household characteristic variables is reported to the nearest whole number. The geometric mean (95% Confidence interval) of the corrected and log-transformed Cotinine distribution for each level of each variable is also reported to the nearest two decimal places. P-values represent test for comparing means between levels of each predictor. P-values less than 0.05 are bolded.
Table A.1c. Distribution of Metabolite Concentrations by Socioeconomic factors, parental disease history and ethnicity, and infant diet.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>% (N)</th>
<th>Geometric mean urinary Cotinine (95% CI), ng/mL</th>
<th>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Household Income</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$0-49,999/year</td>
<td>10.0% (n=201)</td>
<td>0.26 (0.20-0.34)</td>
<td>0.55 (0.43-0.76)</td>
</tr>
<tr>
<td>$50,000-99,999/year</td>
<td>31.3% (n=631)</td>
<td>0.14 (0.12-0.16)</td>
<td>0.26 (0.22-0.29)</td>
</tr>
<tr>
<td>$100,000-149,999/year</td>
<td>26.4% (n=533)</td>
<td>0.09 (0.08-0.11)</td>
<td>0.19 (0.16-0.21)</td>
</tr>
<tr>
<td>$150,000+/year</td>
<td>23.3% (n=469)</td>
<td>0.08 (0.07-0.09)</td>
<td>0.14 (0.12-0.16)</td>
</tr>
<tr>
<td>Prefers to not say</td>
<td>9.1% (n=183)</td>
<td>0.12 (0.10-0.15)</td>
<td>0.28 (0.22-0.36)</td>
</tr>
<tr>
<td><strong>Paternal Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highschool or less</td>
<td>12.4% (n=250)</td>
<td>0.26 (0.20-0.33)</td>
<td>0.59 (0.45-0.78)</td>
</tr>
<tr>
<td>Some Post-Secondary</td>
<td>16.7% (n=336)</td>
<td>0.16 (0.13-0.20)</td>
<td>0.35 (0.28-0.43)</td>
</tr>
<tr>
<td>Completed Post-Secondary</td>
<td>54.5% (n=1099)</td>
<td>0.10 (0.09-0.11)</td>
<td>0.18 (0.16-0.20)</td>
</tr>
<tr>
<td>Masters or PhD</td>
<td>16.5% (n=332)</td>
<td>0.07 (0.06-0.09)</td>
<td>0.14 (0.13-0.17)</td>
</tr>
<tr>
<td><strong>Maternal Education</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Highschool or less</td>
<td>6.6% (n=133)</td>
<td>0.30 (0.21-0.42)</td>
<td>0.75 (0.52-1.08)</td>
</tr>
<tr>
<td>Some Post-Secondary</td>
<td>14.0% (n=282)</td>
<td>0.18 (0.14-0.22)</td>
<td>0.35 (0.28-0.44)</td>
</tr>
<tr>
<td>Completed Post-Secondary</td>
<td>59.3% (n=1196)</td>
<td>0.10 (0.09-0.11)</td>
<td>0.20 (0.18-0.22)</td>
</tr>
<tr>
<td>Masters or PhD</td>
<td>20.1% (n=406)</td>
<td>0.09 (0.08-0.10)</td>
<td>0.16 (0.14-0.19)</td>
</tr>
<tr>
<td><strong>Parental History</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asthma (A)</td>
<td>31.3% (n=660)</td>
<td>0.13 (0.12-0.15)</td>
<td>0.24 (0.21-0.27)</td>
</tr>
<tr>
<td>No Asthma</td>
<td>67.3% (n=1357)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.22 (0.20-0.24)</td>
</tr>
<tr>
<td>Atopy (ATP)</td>
<td>79.9% (n=1611)</td>
<td>0.11 (0.11-0.12)</td>
<td>0.22 (0.20-0.23)</td>
</tr>
<tr>
<td>No Atopy</td>
<td>20.1% (n=406)</td>
<td>0.13 (0.11-0.15)</td>
<td>0.26 (0.22-0.31)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>% (N)</td>
<td>Geometric mean urinary Cotinine (95% CI), ng/mL</td>
<td>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</td>
</tr>
<tr>
<td>------------------------</td>
<td>-------------</td>
<td>-----------------------------------------------</td>
<td>--------------------------------------------------------</td>
</tr>
<tr>
<td><strong>Month of Birth</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>January</td>
<td>8.4% (n=170)</td>
<td>0.11 (0.09-0.14)</td>
<td>0.20 (0.15-0.25)</td>
</tr>
<tr>
<td>February</td>
<td>7.6% (n=153)</td>
<td>0.14 (0.11-0.18)</td>
<td>0.24 (0.18-0.33)</td>
</tr>
<tr>
<td>March</td>
<td>9.9% (n=200)</td>
<td>0.12 (0.10-0.15)</td>
<td>0.24 (0.19-0.30)</td>
</tr>
<tr>
<td>April</td>
<td>8.9% (n=179)</td>
<td>0.12 (0.10-0.16)</td>
<td>0.26 (0.20-0.33)</td>
</tr>
<tr>
<td>May</td>
<td>9.0% (n=182)</td>
<td>0.12 (0.10-0.15)</td>
<td>0.23 (0.18-0.28)</td>
</tr>
<tr>
<td>June</td>
<td>9.1% (n=184)</td>
<td>0.10 (0.08-0.13)</td>
<td>0.23 (0.18-0.29)</td>
</tr>
<tr>
<td>July</td>
<td>8.2% (n=166)</td>
<td>0.12 (0.08-0.16)</td>
<td>0.27 (0.19-0.36)</td>
</tr>
<tr>
<td>August</td>
<td>7.2% (n=145)</td>
<td>0.12 (0.09-0.15)</td>
<td>0.26 (0.19-0.35)</td>
</tr>
<tr>
<td>September</td>
<td>6.9% (n=140)</td>
<td>0.13 (0.09-0.18)</td>
<td>0.21 (0.15-0.30)</td>
</tr>
<tr>
<td>October</td>
<td>7.7% (n=156)</td>
<td>0.09 (0.07-0.12)</td>
<td>0.16 (0.13-0.19)</td>
</tr>
<tr>
<td>November</td>
<td>8.9% (n=179)</td>
<td>0.12 (0.10-0.15)</td>
<td>0.20 (0.16-0.25)</td>
</tr>
<tr>
<td>December</td>
<td>8.1% (n=163)</td>
<td>0.11 (0.08-0.14)</td>
<td>0.23 (0.17-0.30)</td>
</tr>
<tr>
<td><strong>Season of sample Collection</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Summer</td>
<td>24.7% (n=498)</td>
<td>0.13 (0.11-0.15)</td>
<td>0.26 (0.22-0.30)</td>
</tr>
<tr>
<td>Fall</td>
<td>23.2% (n=467)</td>
<td>0.11 (0.09-0.13)</td>
<td>0.24 (0.20-0.27)</td>
</tr>
<tr>
<td>Winter</td>
<td>23.8% (n=481)</td>
<td>0.11 (0.09-0.13)</td>
<td>0.20 (0.17-0.24)</td>
</tr>
<tr>
<td>Spring</td>
<td>28.3% (n=571)</td>
<td>0.12 (0.10-0.13)</td>
<td>0.21 (0.18-0.24)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>% (N)</td>
<td>Geometric mean urinary Cotinine (95% CI), ng/mL</td>
<td>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</td>
</tr>
<tr>
<td>-------------------------</td>
<td>------------</td>
<td>-----------------------------------------------</td>
<td>-------------------------------------------------</td>
</tr>
<tr>
<td><strong>Maternal Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian/White</td>
<td>74.8% (n=1509)</td>
<td>0.11 (0.11-0.12)</td>
<td>0.22 (0.20-0.24)</td>
</tr>
<tr>
<td>East Asian</td>
<td>6.3% (n=127)</td>
<td>0.09 (0.07-0.11)</td>
<td>0.13 (0.11-0.17)</td>
</tr>
<tr>
<td>South East Asian</td>
<td>4.8% (n=97)</td>
<td>0.13 (0.10-0.18)</td>
<td>0.20 (0.14-0.28)</td>
</tr>
<tr>
<td>Multiracial</td>
<td>4.1% (n=82)</td>
<td>0.10 (0.08-0.15)</td>
<td>0.22 (0.15-0.33)</td>
</tr>
<tr>
<td>First Nations</td>
<td>3.4% (n=69)</td>
<td>0.39 (0.23-0.65)</td>
<td>1.02 (0.57-1.82)</td>
</tr>
<tr>
<td>South Asian</td>
<td>2.6% (n=53)</td>
<td>0.09 (0.06-0.13)</td>
<td>0.22 (0.13-0.34)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1.5% (n=31)</td>
<td>0.10 (0.06-0.17)</td>
<td>0.16 (0.10-0.26)</td>
</tr>
<tr>
<td>Black</td>
<td>1.3% (n=27)</td>
<td>0.12 (0.08-0.18)</td>
<td>0.28 (0.17-0.45)</td>
</tr>
<tr>
<td>Middle Eastern</td>
<td>0.9% (n=19)</td>
<td>0.09 (0.05-0.15)</td>
<td>0.24 (0.14-0.42)</td>
</tr>
<tr>
<td>Unknown/Other</td>
<td>0.1% (n=3)</td>
<td>0.07 (0.003-1.46)</td>
<td>0.21 (0.01-3.49)</td>
</tr>
<tr>
<td><strong>Paternal Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian/White</td>
<td>75.4% (n=1521)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.20-0.23)</td>
</tr>
<tr>
<td>East Asian</td>
<td>4.7% (n=95)</td>
<td>0.09 (0.07-0.12)</td>
<td>0.13 (0.10-0.18)</td>
</tr>
<tr>
<td>South East Asian</td>
<td>3.9% (n=79)</td>
<td>0.10 (0.07-0.14)</td>
<td>0.16 (0.12-0.22)</td>
</tr>
<tr>
<td>Multiracial</td>
<td>3.4% (n=68)</td>
<td>0.12 (0.08-0.18)</td>
<td>0.24 (0.17-0.36)</td>
</tr>
<tr>
<td>First Nations</td>
<td>3.7% (n=75)</td>
<td>0.28 (0.18-0.45)</td>
<td>0.65 (0.38-1.09)</td>
</tr>
<tr>
<td>South Asian</td>
<td>3.5% (n=70)</td>
<td>0.11 (0.08-0.15)</td>
<td>0.22 (0.15-0.32)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1.5% (n=31)</td>
<td>0.12 (0.06-0.25)</td>
<td>0.23 (0.11-0.48)</td>
</tr>
<tr>
<td>Black</td>
<td>2.1% (n=45)</td>
<td>0.18 (0.09-0.33)</td>
<td>0.65 (0.32-1.33)</td>
</tr>
<tr>
<td>Middle Eastern</td>
<td>1.0% (n=20)</td>
<td>0.08 (0.05-0.12)</td>
<td>0.19 (0.12-0.30)</td>
</tr>
<tr>
<td>Unknown/Other</td>
<td>0.6% (n=13)</td>
<td>0.25 (0.07-0.85)</td>
<td>0.52 (0.12-2.17)</td>
</tr>
<tr>
<td>Characteristic</td>
<td>% (N)</td>
<td>Geometric mean urinary Cotinine (95% CI), ng/mL</td>
<td>Geometric mean urinary Hydroxycotinine (95% CI), ng/mL</td>
</tr>
<tr>
<td>--------------------------------</td>
<td>-------</td>
<td>-------------------------------------------------</td>
<td>-----------------------------------------------------</td>
</tr>
<tr>
<td>Maternal Age at Enrolment</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>17 to 23 years old</td>
<td>3.8%  (n=77)</td>
<td>0.42 (0.28-0.64)</td>
<td>1.01 (0.64-1.60)</td>
</tr>
<tr>
<td>24 to 30 years old</td>
<td>32.2% (n=649)</td>
<td>0.14 (0.12-0.16)</td>
<td>0.29 (0.25-0.33)</td>
</tr>
<tr>
<td>31 to 35 years old</td>
<td>41.9% (n=845)</td>
<td>0.10 (0.09-0.11)</td>
<td>0.19 (0.17-0.21)</td>
</tr>
<tr>
<td>36-46 years old</td>
<td>22.1% (n=446)</td>
<td>0.09 (0.08-0.11)</td>
<td>0.17 (0.15-0.20)</td>
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<tr>
<td>Study Centre</td>
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<tr>
<td>Vancouver</td>
<td>26.7% (n=539)</td>
<td>0.10 (0.09-0.12)</td>
<td>0.16 (0.14-0.18)</td>
</tr>
<tr>
<td>Edmonton</td>
<td>19.9% (n=402)</td>
<td>0.13 (0.11-0.15)</td>
<td>0.28 (0.23-0.34)</td>
</tr>
<tr>
<td>Winnipeg, Morden, or Winkler</td>
<td>30.9% (n=624)</td>
<td>0.14 (0.12-0.16)</td>
<td>0.29 (0.25-0.34)</td>
</tr>
<tr>
<td>Toronto</td>
<td>22.4% (n=452)</td>
<td>0.09 (0.08-0.10)</td>
<td>0.20 (0.17-0.22)</td>
</tr>
<tr>
<td>Breastfeeding Status</td>
<td></td>
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<tr>
<td>None</td>
<td>12.0% (n=243)</td>
<td>0.16 (0.13-0.19)</td>
<td>0.29 (0.23-0.36)</td>
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<tr>
<td>Partial</td>
<td>25.8% (n=520)</td>
<td>0.12 (0.10-0.14)</td>
<td>0.24 (0.20-0.28)</td>
</tr>
<tr>
<td>Exclusive</td>
<td>62.2% (n=1254)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.23)</td>
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<tr>
<td>Infant’s 3-month Diet</td>
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</tr>
<tr>
<td>Breastfeeding Only</td>
<td>62.2% (n=1254)</td>
<td>0.11 (0.10-0.12)</td>
<td>0.21 (0.19-0.23)</td>
</tr>
<tr>
<td>Breastfeeding and Formula</td>
<td>24.2% (n=489)</td>
<td>0.12 (0.10-0.14)</td>
<td>0.24 (0.20-0.28)</td>
</tr>
<tr>
<td>Breastfeeding and Solid Food</td>
<td>0.9% (n=18)</td>
<td>0.13 (0.04-0.37)</td>
<td>0.34 (0.11-1.02)</td>
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<tr>
<td>Formula Only</td>
<td>11.2% (n=226)</td>
<td>0.15 (0.12-0.19)</td>
<td>0.27 (0.21-0.34)</td>
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<td>Formula and Solid Food</td>
<td>0.8% (n=17)</td>
<td>0.27 (0.09-0.77)</td>
<td>0.71 (0.23-2.17)</td>
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<tr>
<td>Breastfeeding, Formula, and Solid Food</td>
<td>0.6% (n=13)</td>
<td>0.12 (0.05-0.28)</td>
<td>0.20 (0.06-0.63)</td>
</tr>
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</table>

The proportion and crude number of sample subjects that corresponds to each level of household characteristic variables is reported to the nearest whole number. The geometric mean (95% Confidence interval) of the corrected and log-transformed Cotinine distribution for each level of each variable is also reported to the nearest two decimal places. P-values represent tests for comparing means between levels of each predictor. P-values less than 0.05 are bolded.
Figure A.2a. Variable importance plot for cotinine concentration
Variables with higher importance scores (top right) in predicting the distribution of the log-transformed \( \text{trans-3'-hydroxycotinine} \) concentration (ng/mL) were found through random forest regression. Variables related to second-hand smoke (green), household characteristics or third-hand smoke reservoirs (blue), and other (red).
Figure A.2b.  Variable importance plot for trans-3’-hydroxycotinine concentration
Variables with higher importance scores (top right) in predicting the distribution of the log-transformed trans-3’-hydroxycotinine concentration (ng/mL) were found through random forest regression. Variables related to second-hand smoke (green), household characteristics or third-hand smoke reservoirs (blue), and other (red).
<table>
<thead>
<tr>
<th>Predictor</th>
<th>Variable Importance Score (%MSE)</th>
<th>R² from Bivariate Analysis (%)</th>
<th>Outcome: Cotinine</th>
<th>Variable Importance Score (%MSE)</th>
<th>R² from Bivariate Analysis (%)</th>
<th>Outcome: Hydroxycotinine</th>
</tr>
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<tbody>
<tr>
<td>Maternal Smoking Status Prior to Pregnancy</td>
<td>23.10</td>
<td>18.8</td>
<td>22.49</td>
<td>22.9</td>
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<tr>
<td>Cigarettes smoked daily at home in early life</td>
<td>18.92</td>
<td>14.4</td>
<td>19.96</td>
<td>18.3</td>
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</tr>
<tr>
<td>Frequency of maternal smoking during pregnancy</td>
<td>18.70</td>
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<td>Days pregnant mother has been exposed to tobacco smoke in the past 2 weeks</td>
<td>18.47</td>
<td>15.3</td>
<td>17.49</td>
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<tr>
<td>Week of gestation mother quit smoking during pregnancy</td>
<td>16.93</td>
<td>9.3</td>
<td>15.35</td>
<td>11.8</td>
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</tr>
<tr>
<td>Average Cigarettes Smoked in Pregnancy</td>
<td>16.15</td>
<td>15.6</td>
<td>14.76</td>
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<tr>
<td>Average Daily Cigarettes Smoked at Home in Pregnancy</td>
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<td>14.3</td>
<td>15.74</td>
<td>17.7</td>
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<td>Household Smoking since birth</td>
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<tr>
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<td>14.8</td>
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<tr>
<td>Average Daily Cigarettes during Pregnancy before quitting</td>
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<td>8.82</td>
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<tr>
<td>Household Smoking during pregnancy</td>
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<td>11.3</td>
<td>13.31</td>
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<td>Household smoking indoors during pregnancy</td>
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<td>13.61</td>
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<td>Household smoking outside since birth</td>
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<td>11.77</td>
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<td>Outcome: Hydroxycotinine</td>
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<td><strong>Variable Importance Score (%MSE)</strong></td>
<td><strong>R^2 from Bivariate Analysis (%)</strong></td>
<td><strong>Variable Importance Score (%MSE)</strong></td>
<td><strong>R^2 from Bivariate Analysis (%)</strong></td>
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<tr>
<td>Household smoking near window or in garage during pregnancy</td>
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<td>11.5</td>
<td>12.26</td>
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<td>Household Income</td>
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<tr>
<td>Hours of exposure, past 2 weeks of pregnancy</td>
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<td>Frequency of a household smoker in pregnancy</td>
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<td>Cut down smoking during pregnancy</td>
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<td>Dwelling Type, 7 groups</td>
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<tr>
<td>Days baby exposed in past week</td>
<td>7.44</td>
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<td>Daily cigarettes before cutting down in pregnancy</td>
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<td>Area rug in basement</td>
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<td>Week Reduced Smoking during pregnancy</td>
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<td>Age Started Smoking</td>
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<td>Number of Bedrooms</td>
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<td>Outcome: Hydroxycotinine</td>
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<td>R² from Bivariate Analysis (%)</td>
<td>Variable Importance Score (%MSE)</td>
<td>R² from Bivariate Analysis (%)</td>
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<td>Carpeted basement</td>
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<td>Carpeted living space</td>
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<td>Week gestation reduced smoking, numeric</td>
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<td>Hours baby exposed in past week of life</td>
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<td>Ever smoked for 1 year</td>
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<td>Outcome: Hydroxycotinine</td>
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<td>R² from Bivariate Analysis (%)</td>
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<td>R² from Bivariate Analysis (%)</td>
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</tr>
<tr>
<td>Household smoking near a window or in the garage, early life</td>
<td>0.75</td>
<td>3.5</td>
<td>1.87</td>
<td>4.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Birthweight</td>
<td>0.66</td>
<td>0.07</td>
<td>-4.32</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Month of birth</td>
<td>-0.06</td>
<td>0.4</td>
<td>6.47</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paternal ethnicity</td>
<td>-0.29</td>
<td>1.6</td>
<td>4.66</td>
<td>3.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folate Supplements, numeric</td>
<td>-0.71</td>
<td>0.02</td>
<td>-2.64</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household smoking inside, early life</td>
<td>-0.76</td>
<td>1.5</td>
<td>0.12</td>
<td>1.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Folate Supplements</td>
<td>-0.91</td>
<td>0.06</td>
<td>-3.86</td>
<td>0.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Child’s sex</td>
<td>-1.16</td>
<td>0.03</td>
<td>-0.15</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carpeted kitchen</td>
<td>-2.92</td>
<td>0.1</td>
<td>-0.72</td>
<td>&lt;0.001</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Predictor Variables are ordered from highest (top) to lowest (bottom) variable importance against log-transformed cotinine concentration, as determined through random forest regression. Importance scores are reported to the nearest 2 decimal places and reflect either the percentage of increase in mean squared error (%MSE) the average model will incur should the predictor be excluded from the model. R² values are reported as a percentage based on bivariate linear regression analysis between a predictor variable and the log-transformed metabolite concentration.
Figure A.3a. Distribution of log-transformed cotinine concentration following imputation of concentrations below detection

The post-imputation distribution ranges from -10.49 to 7.41, which equates to an original concentration range from 0.0007 to 170.0 ng/mL. The imputed cotinine concentrations ranged from -10.49 to -5.06 which equates to an original concentration of 0.0007 to 0.02997 ng/mL, consistent with our range of 0-0.03 ng/mL. The median concentration of the original cotinine concentrations was 0.016 ng/mL, with a mean of 0.0161 ng/mL. The level of detection was 0.03 ng/mL.
Figure A.3b. Distribution of log-transformed Hydroxycotinine concentration following imputation of concentrations below detection

The post-imputation distribution ranges from -8.49 to 10.30, which equates to an original concentration range from 0.0028 to 1260.0 ng/mL. The imputed hydroxycotinine concentrations ranged from a raw concentration of 0.0004 to 0.0299 ng/mL, consistent with our range of 0-0.03 ng/mL. The Median concentration of the original cotinine concentrations was 0.0141 ng/mL, with a mean of 0.0150 ng/mL. The level of detection was 0.03 ng/mL.
### Table A.3. Predictor data dictionary

<table>
<thead>
<tr>
<th>Predictor</th>
<th>Description</th>
<th>Levels or range of responses</th>
<th>Collection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal Smoking Status Prior to Pregnancy</td>
<td>The mother’s smoking status prior the pregnancy</td>
<td>3 groups; • Never smoked • Quit prior to the pregnancy • Did not quit prior to the pregnancy</td>
<td>Prenatal Maternal Health Questionnaire</td>
</tr>
<tr>
<td>Frequency of maternal smoking during pregnancy</td>
<td>How often the mother smoked during her pregnancy</td>
<td>2 levels; • Never • Occasionally or Regularly</td>
<td>Prenatal Maternal Health Questionnaire</td>
</tr>
<tr>
<td>Days pregnant mother has been exposed to tobacco smoke in the past 2 weeks</td>
<td>Average number of days the pregnant mother was exposed to tobacco smoke in the past 2 weeks</td>
<td>Numerical range from 0-14.</td>
<td>Home Environment Questionnaire, completed during pregnancy</td>
</tr>
<tr>
<td>Week of gestation mother quit smoking during pregnancy</td>
<td>Week of gestation that the mother quit smoking during her pregnancy</td>
<td>Numerical range from 0-25.</td>
<td>Prenatal Maternal Health Questionnaire</td>
</tr>
<tr>
<td>Average Cigarettes Smoked in Pregnancy</td>
<td>Average number of cigarettes the pregnancy mother smoked per day</td>
<td>3 groups; • None • Less than 10/day • 10 to 20/day</td>
<td>Prenatal Maternal Health Questionnaire</td>
</tr>
<tr>
<td>Average Daily Cigarettes Smoked at Home in Pregnancy</td>
<td>The average number of cigarettes/cigars/pipes smoked at the household per day</td>
<td>5 groups; • None • Less than 1/day • 1-5/day • 6-10 /day • More than 10/day</td>
<td>Prenatal Maternal Health Questionnaire</td>
</tr>
<tr>
<td>Household Smoking since birth</td>
<td>Whether or not anyone smokes at the home since the child’s birth</td>
<td>2 levels; • No • Yes</td>
<td>Home Environment Questionnaire, completed by parent(s) at 3-4 months of age</td>
</tr>
<tr>
<td>Average Daily Cigarettes during Pregnancy before quitting</td>
<td>Average number of cigarettes the pregnancy mother smoked per day prior to quitting</td>
<td>4 groups; • Never Smoke • Less than 5/day • 5-10/day • 10-25 /day</td>
<td>Prenatal Maternal Health Questionnaire</td>
</tr>
<tr>
<td>Predictor</td>
<td>Description</td>
<td>Levels or range of responses</td>
<td>Collection method</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>-----------------------------------------------------------------------------</td>
<td>-----------------------------------------------------------------------------------------------</td>
<td>---------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Household Income</td>
<td>Parental response to “What is the best estimate of total income, before taxes and deductions, of all household members, from all sources in the past 12 months.”</td>
<td>5 levels;</td>
<td>Socioeconomic Status Questionnaire* *completed during pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• $0-49,999/year • $50,000-99,999/year • $100,000-149,999/year • $150,000+/year • Prefers not to say</td>
<td></td>
</tr>
<tr>
<td>Breastfeeding Status</td>
<td>Status of infants breastfeeding at 3 months of age</td>
<td>3 levels;</td>
<td>Determined using a combination of the birth and nutrition questionnaires</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Not breastfed (no recorded initiation of breastfeeding in hospital) • Partially breastfed (any combination of breastfeeding and supplementation) • Exclusively breastfed until any supplement (including formula, other fluid (non-human milk, juice) or food) is introduced.)</td>
<td></td>
</tr>
<tr>
<td>Centre</td>
<td>Study Centre location of the subject</td>
<td>4 groups;</td>
<td>Home Environment Questionnaire completed by parents when child is 3-4 months of age</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Vancouver • Edmonton • Winnipeg, Morden, and Winkler • Toronto</td>
<td></td>
</tr>
<tr>
<td>Frequency of a household smoker in pregnancy</td>
<td>Whether and how frequent anyone smoked inside the home during pregnancy</td>
<td>3 levels;</td>
<td>Home Environment Questionnaire, completed during pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• None • Occasional • Regular</td>
<td></td>
</tr>
<tr>
<td>Parental Asthma</td>
<td>Whether or not at least one parent has a history or current diagnosis of asthma. If one parent was missing, and the other had a positive response, the child was positive. If both had no response, or if one was a no and the other missing, the child did not have a history.</td>
<td>2 levels;</td>
<td>Derived from Prenatal Health Questionnaire</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• No • Yes</td>
<td></td>
</tr>
</tbody>
</table>

109
<table>
<thead>
<tr>
<th>Predictor</th>
<th>Description</th>
<th>Levels or range of responses</th>
<th>Collection method</th>
</tr>
</thead>
<tbody>
<tr>
<td>Paternal Education</td>
<td>Highest level of education achieved by the father</td>
<td>4 levels;</td>
<td>Socioeconomic Status Questionnaire*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Highschool or less</td>
<td>*completed during pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Some post-secondary</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Completed post-secondary</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Masters of PhD</td>
<td></td>
</tr>
<tr>
<td>Dwelling Type, 3 groups</td>
<td>The type of dwelling that best described the subject's home</td>
<td>3 groups;</td>
<td>Socioeconomic Status Questionnaire*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Mobile home/trailer/Other</td>
<td>*completed during pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Single Family (detached or semi-detached)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Multi-family/Apartment (any number of levels)</td>
<td></td>
</tr>
<tr>
<td>Week gestation</td>
<td>Week of gestation that the mother reduced smoking during her pregnancy</td>
<td>Numeric range from 0-21</td>
<td>Prenatal Maternal Health Questionnaire</td>
</tr>
<tr>
<td>reduced smoking</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home Ownership</td>
<td>Questionnaire response to &quot;Does your family own or rent your house/apartment?&quot;</td>
<td>2 levels;</td>
<td>Socioeconomic Status Questionnaire*</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Rent</td>
<td>*completed during pregnancy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Own</td>
<td></td>
</tr>
<tr>
<td>Carpeted child's room</td>
<td>Research staff indication of whether or not the child's bedroom had installed</td>
<td>2 levels;</td>
<td>Home assessment Questionnaire, completed by research staff at 3-4 months of age</td>
</tr>
<tr>
<td></td>
<td>carpet as the type of flooring.</td>
<td>• No</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Yes</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• No child-specific room</td>
<td></td>
</tr>
<tr>
<td>Collection Season</td>
<td>Season of urine sample collection. Derived from the month when urine samples</td>
<td>4 levels, set as per Northern Meteorological Seasons;</td>
<td>Urinary biomarker sampling collected at the 3-4-month of age home assessment</td>
</tr>
<tr>
<td></td>
<td>were collected.</td>
<td>• Spring</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Summer</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Fall</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>• Winter</td>
<td></td>
</tr>
</tbody>
</table>

This table offers an explanation of how each of the variables in our final predictor models (Tables 3a and 3b of main paper) were organized and derived.
Table A4. Cotinine Multivariable Linear Regression Model and Multiplicative Change in log-transformed Cotinine concentrations

<table>
<thead>
<tr>
<th>Predictor variables (n=13)</th>
<th>Cotinine Model Coefficients (95% CI)</th>
<th>Multiplicative Change in Concentration (95% CI)</th>
<th>R² (%) of Model</th>
<th>Multivariable Cotinine Model Coefficients (95% CI)*</th>
<th>Multiplicative Change in Concentration (95% CI)*</th>
<th>Model R² (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mother’s Smoking Prior to Pregnancy</td>
<td></td>
<td>18.83</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Did not quit smoking prior to pregnancy vs. Never Smoked</td>
<td>4.30 (3.91, 4.69)</td>
<td>19.72 (15.05-24.86)</td>
<td>1.24 (0.58, 1.90)</td>
<td>2.236 (1.49-3.74)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Quit Smoking prior to pregnancy vs. Never Smoked</td>
<td>0.42 (0.18, 0.67)</td>
<td>1.34 (1.13-1.59)</td>
<td>0.17 (-0.06, 0.40)</td>
<td>1.13 (0.96-1.32)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mother’s Reported Smoking Frequency in Pregnancy</td>
<td></td>
<td>16.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Smoked daily or occasionally vs. Never Smoked</td>
<td>4.30 (3.87, 4.74)</td>
<td>19.76 (14.66-26.64)</td>
<td>2.20 (1.53, 2.87)</td>
<td>4.59 (2.89-7.29)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Days Exposed to Cigarette Smoke in past 2 weeks during pregnancy</td>
<td>15.25</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>0.29 (0.26, 0.32)</td>
<td>1.22 (1.20-1.25)</td>
<td>0.07 (0.03, 0.10)</td>
<td>1.05 (1.02-1.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week of gestation when mother quit smoking</td>
<td></td>
<td>7.79</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>0.33 (0.28, 0.38)</td>
<td>1.26 (1.21-1.30)</td>
<td>0.14 (0.08, 0.21)</td>
<td>1.10 (1.05-1.15)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Week of gestation when mother cut down smoking</td>
<td></td>
<td>8.31</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Continuous</td>
<td>0.49 (0.42, 0.56)</td>
<td>1.40 (1.34-1.47)</td>
<td>-0.08 (-0.17, -0.01)</td>
<td>0.95 (0.89-1.01)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predictor variables (n=13)</td>
<td>Cotinine Model Coefficients (95% CI)</td>
<td>Multiplicative Change in Concentration (95% CI)</td>
<td>( R^2 ) (%) of Model</td>
<td>Multivariable Cotinine Model Coefficients (95% CI)*</td>
<td>Multiplicative Change in Concentration (95% CI)*</td>
<td>Model R^2 (%)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>--------------------------------------</td>
<td>-----------------------------------------------</td>
<td>------------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Average Daily Cigarettes Smoked at the Home during pregnancy</td>
<td>14.27</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Less than 1/day vs None</td>
<td>3.63 (3.11, 4.15)</td>
<td>12.41 (8.66-17.79)</td>
<td>0.64 (0.03, 1.25)</td>
<td>1.56 (1.02-2.37)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5/day vs None</td>
<td>0.33 (-0.18, 0.83)</td>
<td>1.25 (0.89-1.78)</td>
<td>-0.54 (-1.03, -0.06)</td>
<td>0.69 (0.49-0.96)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-10/day vs None</td>
<td>0.38 (-0.23, 0.99)</td>
<td>1.30 (0.85-1.98)</td>
<td>0.15 (-0.40, 0.70)</td>
<td>1.11 (0.76-1.63)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11+/day vs None</td>
<td>-0.19 (-0.74, 0.35)</td>
<td>0.88 (0.60-1.28)</td>
<td>-0.11 (-0.61, 0.39)</td>
<td>0.93 (0.66-1.31)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Has anyone smoked at the baby's home since their birth?</td>
<td>10.97</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes vs. No</td>
<td>2.39 (2.10, 2.69)</td>
<td>5.26 (4.28-6.46)</td>
<td>0.31 (-0.19, 0.80)</td>
<td>1.24 (0.88-1.74)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Average Daily Cigarettes Smoked at the Home since child's birth</td>
<td>14.37</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-5/day vs. None</td>
<td>3.61 (3.07, 4.15)</td>
<td>12.23 (8.41, 17.77)</td>
<td>0.77 (0.10, 1.43)</td>
<td>1.70 (1.08, 2.69)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6-10/day vs. None</td>
<td>-0.48 (-1.05, 0.08)</td>
<td>0.72 (0.48, 1.06)</td>
<td>0.03 (-0.54, 0.60)</td>
<td>1.02 (0.69, 1.51)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>11+/day vs. None</td>
<td>0.01 (-0.57, 0.60)</td>
<td>1.01 (0.67, 1.51)</td>
<td>0.17 (-0.38, 0.71)</td>
<td>1.12 (0.77, 1.64)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Breastfeeding status at 3 months</td>
<td>0.48</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partially vs. Exclusively Breastfed</td>
<td>0.36 (0.13, 0.59)</td>
<td>1.29 (1.10-1.51)</td>
<td>-0.28 (-0.48, -0.08)</td>
<td>0.82 (0.72-0.95)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Never vs. Exclusively Breastfed</td>
<td>0.13 (-0.08, 0.34)</td>
<td>1.10 (0.95-1.27)</td>
<td>-0.09 (-0.27, 0.09)</td>
<td>0.94 (0.83-1.07)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dwelling Type</td>
<td>1.23</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Predictor variables (n=13)</td>
<td>Cotinine Model Coefficients (95% CI)</td>
<td>Multiplicative Change in Concentration (95% CI)</td>
<td>$R^2$ (%) of Model</td>
<td>Multivariable Cotinine Model Coefficients (95% CI)*</td>
<td>Multiplicative Change in Concentration (95% CI)*</td>
<td>Model $R^2$ (%)</td>
</tr>
<tr>
<td>---------------------------</td>
<td>-------------------------------------</td>
<td>-----------------------------------------------</td>
<td>-----------------</td>
<td>---------------------------------</td>
<td>-----------------------------------------------</td>
<td>----------------</td>
</tr>
<tr>
<td>Multi-family vs. single-family Home</td>
<td>0.83 (0.18, 1.47)</td>
<td>1.77 (1.13-2.77)</td>
<td>0.19 (-0.36, 0.75)</td>
<td>1.14 (0.78-1.68)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trailer/other vs. single-family Home</td>
<td>0.04 (-0.37, 0.44)</td>
<td>1.03 (0.77-1.36)</td>
<td>-0.16 (-0.51, 0.19)</td>
<td>0.89 (0.70-1.14)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Household Income</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4.60</td>
</tr>
<tr>
<td>$50,000-99,999/year vs. &lt;$50,000/year</td>
<td>-0.96 (-1.26, -0.65)</td>
<td>0.52 (0.42-0.64)</td>
<td>-0.45 (-0.72, -0.18)</td>
<td>0.73 (0.61-0.88)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$100,000-149,999/year vs. &lt;$50,000/year</td>
<td>0.90 (0.62, 1.18)</td>
<td>1.87 (1.54-2.27)</td>
<td>0.31 (-0.06, 0.56)</td>
<td>1.24 (1.04-1.47)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$150,000+/year vs. &lt;$50,000/year</td>
<td>0.18 (-0.05, 0.41)</td>
<td>1.13 (0.97-1.33)</td>
<td>0.08 (-0.12, 0.27)</td>
<td>1.05 (0.92-1.21)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prefers not to say vs. &lt;$50,000/year</td>
<td>0.06 (-0.14, 0.26)</td>
<td>1.04 (0.91-1.20)</td>
<td>-0.06 (-0.23, 0.11)</td>
<td>0.96 (0.85-1.08)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Paternal Education</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5.29</td>
</tr>
<tr>
<td>Some post-secondary vs. Highschool or less</td>
<td>-1.37 (-1.63, -1.11)</td>
<td>0.39 (0.32-0.46)</td>
<td>-0.37 (-0.62, -0.13)</td>
<td>0.77 (0.65-0.92)</td>
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<tr>
<td>Completed post-secondary vs. Highschool or less</td>
<td>0.13 (-0.11, 0.36)</td>
<td>1.09 (0.93-1.29)</td>
<td>-0.16 (-0.37, 0.04)</td>
<td>0.89 (0.77-1.03)</td>
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<tr>
<td>Masters or PhD vs. Highschool or less</td>
<td>0.04 (-0.16, 0.25)</td>
<td>1.03 (0.89-1.19)</td>
<td>0.05 (-0.13, 0.23)</td>
<td>1.04 (0.92-1.17)</td>
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<tr>
<td>Parental History of Asthma</td>
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<tr>
<td>Yes vs. No</td>
<td>0.25 (0.03, 0.47)</td>
<td>1.19 (1.02-1.39)</td>
<td>0.22 (0.03, 0.41)</td>
<td>1.16 (1.02-1.32)</td>
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</table>

Estimated change in log-transformed metabolite level by predictors in unadjusted regression models (95% confidence intervals), as well as the coinciding $R^2$ values are shown to the nearest second decimal place. * Estimated change in log-transformed metabolite level by predictors in adjusted final regression models (95% confidence intervals), as well as the coinciding $R^2$ values are shown to the nearest second decimal place.
Table A5.  *Trans*-3'-Hydroxycotinine Multivariable Linear Regression Model and Multiplicative Change in log-transformed *trans*-3'-Hydroxycotinine concentrations

<table>
<thead>
<tr>
<th>Predictor variables (n=19)</th>
<th>3HC Model Coefficients (95% CI)</th>
<th>Multiplicative Change in Concentration (95% CI)</th>
<th>R² (%) of Multivariable 3HC Model Coefficients (95% CI)*</th>
<th>Multiplicative Change in Concentration (95% CI)</th>
<th>Model R² (%)</th>
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<tr>
<td>Mother’s Smoking Prior to Pregnancy</td>
<td>22.90</td>
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<tr>
<td>Did not quit smoking prior vs. Never Smoked</td>
<td>4.95 (4.55, 5.35)</td>
<td>30.86 (23.40-40.68)</td>
<td>1.99 (1.09, 2.88)</td>
<td>3.97 (2.13-7.37)</td>
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</tr>
<tr>
<td>Quit Smoking prior vs. Never Smoked</td>
<td>0.68 (0.43, 0.93)</td>
<td>1.60 (1.35-1.90)</td>
<td>0.45 (0.23, 0.68)</td>
<td>1.37 (1.17-1.60)</td>
<td></td>
</tr>
<tr>
<td>Average Cigarettes Smoked Daily Prior to Quitting During Pregnancy</td>
<td>9.11</td>
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</tr>
<tr>
<td>Less than 5/day vs. Never Smoked</td>
<td>3.61 (2.91, 4.31)</td>
<td>12.20 (7.49-19.88)</td>
<td>0.65 (-0.32, 1.62)</td>
<td>1.57 (0.80-3.07)</td>
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<tr>
<td>5 to 10/day vs. vs. Never Smoked</td>
<td>-0.54 (-1.31, 0.23)</td>
<td>0.69 (0.40-1.17)</td>
<td>1.60 (0.78, 2.43)</td>
<td>3.03 (1.72-5.38)</td>
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<tr>
<td>11 to 25/day vs. Never Smoked</td>
<td>0.50 (-0.32, 1.32)</td>
<td>1.42 (0.80-2.50)</td>
<td>-0.21 (-0.91, 0.49)</td>
<td>0.86 (0.53-1.40)</td>
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<td>Mother’s Reported Smoking Frequency in Pregnancy</td>
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<tr>
<td>Smoked daily or occasionally vs. Never Smoked</td>
<td>4.80 (4.35, 5.24)</td>
<td>27.77 (20.39-37.81)</td>
<td>3.13 (1.16, 5.10)</td>
<td>8.73 (2.22-34.21)</td>
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</tr>
<tr>
<td>Average Cigarettes Smoked by Mother in early pregnancy</td>
<td>17.56</td>
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<tr>
<td>Less than 10/day vs. None</td>
<td>5.39 (4.67, 6.11)</td>
<td>42.01 (25.51-69.20)</td>
<td>-0.60 (-2.72, 1.53)</td>
<td>0.66 (0.15-2.88)</td>
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<td>10-20/day vs. None</td>
<td>-1.97 (-2.75, -1.18)</td>
<td>0.26 (0.15-0.44)</td>
<td>1.58 (0.25, 2.91)</td>
<td>2.98 (1.19-7.50)</td>
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<tr>
<td>Predictor variables (n=19)</td>
<td>3HC Model Coefficients (95% CI)</td>
<td>Multiplicative Change in Concentration (95% CI)</td>
<td>R² (%) of Multivariable 3HC Model Coefficients (95% CI)*</td>
<td>Multiplicative Change in Concentration (95% CI)</td>
<td>Model R² (%)</td>
</tr>
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</tr>
<tr>
<td>Days Exposed to Cigarette Smoke in past 2 weeks during pregnancy</td>
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<tr>
<td>Continuous</td>
<td>0.33 (0.30, 0.36)</td>
<td>1.26 (1.23-1.29)</td>
<td>0.05 (0.01, 0.10)</td>
<td>1.04 (1.01-1.07)</td>
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<tr>
<td>Mother had any recent tobacco smoke exposure during pregnancy</td>
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<tr>
<td>Recent exposure</td>
<td>1.98 (1.73, 2.23)</td>
<td>3.94 (3.31-4.68)</td>
<td>0.24 (-0.07, 0.54)</td>
<td>1.17 (0.95-1.46)</td>
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<tr>
<td>Week of gestation when mother quit smoking</td>
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</tr>
<tr>
<td>Continuous</td>
<td>0.39 (0.34, 0.44)</td>
<td>1.30 (1.26-1.35)</td>
<td>0.16 (0.08, 0.25)</td>
<td>1.12 (1.06-1.19)</td>
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</tr>
<tr>
<td>Did anyone smoke at the baby’s home during pregnancy?</td>
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<tr>
<td>Occasionally vs. None</td>
<td>3.54 (2.74, 4.33)</td>
<td>11.60 (6.68-20.12)</td>
<td>0.16 (-0.54, 0.87)</td>
<td>1.12 (0.69-1.83)</td>
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<tr>
<td>Regularly vs. None</td>
<td>4.93 (4.00, 5.86)</td>
<td>30.41 (15.87-58.25)</td>
<td>0.54 (-0.37, 1.44)</td>
<td>1.45 (0.78-2.71)</td>
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<tr>
<td>Average Daily Cigarettes Smoked at the Home during pregnancy</td>
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<td></td>
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<td></td>
</tr>
<tr>
<td>Less than 1/day vs None</td>
<td>4.07 (3.54, 4.61)</td>
<td>16.85 (11.64-24.37)</td>
<td>0.21 (-0.44, 0.86)</td>
<td>1.16 (0.74-1.81)</td>
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<tr>
<td>1-5/day vs None</td>
<td>0.24 (-0.28, 0.75)</td>
<td>1.18 (0.82-1.69)</td>
<td>-0.85 (-1.36, -0.34)</td>
<td>0.56 (0.39-0.79)</td>
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<tr>
<td>6-10/day vs None</td>
<td>0.61 (-0.02, 1.23)</td>
<td>1.52 (1.00-2.34)</td>
<td>0.23 (-0.33, 0.79)</td>
<td>1.17 (0.79-1.73)</td>
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<tr>
<td>11+/day vs None</td>
<td>-0.13 (-0.69, 0.43)</td>
<td>0.91 (0.62-1.34)</td>
<td>0.09 (-0.40, 0.58)</td>
<td>1.06 (0.76-1.50)</td>
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</tr>
<tr>
<td>Predictor variables (n=19)</td>
<td>3HC Model Coefficients (95% CI)</td>
<td>Multiplicative Change in Concentration (95% CI)</td>
<td>R² (%) of Multivariable 3HC Model Coefficients (95% CI)*</td>
<td>Multiplicative Change in Concentration (95% CI)</td>
<td>Model R² (%) 40.89</td>
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<tr>
<td>Has anyone smoked at the baby’s home since their birth?</td>
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<tr>
<td>Yes vs. No</td>
<td>2.96 (2.65, 3.26)</td>
<td>7.77 (6.30-9.60)</td>
<td>0.70 (0.22, 1.19)</td>
<td>1.63 (1.16-2.28)</td>
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<tr>
<td>Average Daily Cigarettes Smoked at the Home since child’s birth</td>
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</tr>
<tr>
<td>1-5/day vs. None</td>
<td>4.03 (3.48, 4.58)</td>
<td>16.37 (11.17-24.00)</td>
<td>0.41 (-0.24, 1.07)</td>
<td>1.33 (0.85-2.10)</td>
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<td>6-10/day vs. None</td>
<td>-0.80 (-1.37, -0.22)</td>
<td>0.58 (0.39-0.86)</td>
<td>-0.12 (-0.68, 0.45)</td>
<td>0.92 (0.62-1.36)</td>
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<tr>
<td>11+/day vs. None</td>
<td>0.11 (-0.49, 0.71)</td>
<td>1.08 (0.71-1.63)</td>
<td>0.24 (-0.30, 0.78)</td>
<td>1.18 (0.81-0.72)</td>
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<td>Carpeting in the home</td>
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<td>1.53</td>
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<td>Child’s room is not carpeted vs. No child-specific room</td>
<td>-0.59 (-0.81, -0.38)</td>
<td>0.66 (0.57-0.77)</td>
<td>-0.18 (-0.37, -0.0)</td>
<td>0.88 (0.78-1.00)</td>
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<tr>
<td>Child’s room is carpeted vs. No child-specific room</td>
<td>0.27 (0.09, 0.46)</td>
<td>1.21 (1.06-1.37)</td>
<td>-0.05 (-0.20, 0.11)</td>
<td>0.97 (0.87-1.08)</td>
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<td>Breastfeeding status at 3 months</td>
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<td>0.39</td>
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<td>Partially vs. Exclusively Breastfed</td>
<td>0.32 (0.08, 0.56)</td>
<td>1.25 (1.06-1.48)</td>
<td>-0.52 (-0.72, -0.32)</td>
<td>0.70 (0.61-0.80)</td>
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<tr>
<td>Not vs. Exclusively Breastfed</td>
<td>0.03 (-0.19, 0.25)</td>
<td>1.02 (0.88-1.19)</td>
<td>-0.29 (-0.47, -0.12)</td>
<td>0.82 (0.72-0.92)</td>
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<tr>
<td>Predictor variables (n=19)</td>
<td>3HC Model Coefficients (95% CI)</td>
<td>Multiplicative Change in Concentration (95% CI)</td>
<td>Multivariable 3HC Model Coefficients (95% CI)*</td>
<td>R² (%) of Multivariable 3HC Model</td>
<td>Multiplicative Change in Concentration (95% CI)</td>
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<td>Dwelling Type</td>
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<td>Multi-family vs. single-family Home</td>
<td>0.99 (0.31, 1.66)</td>
<td>1.98 (1.24-3.17)</td>
<td>0.30 (-0.25, 0.84)</td>
<td>1.23 (0.84-1.79)</td>
<td>1.98 (1.24-3.17)</td>
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<tr>
<td>Trailer/other vs. single-family Home</td>
<td>0.12 (-0.31, 0.54)</td>
<td>1.08 (0.81-1.46)</td>
<td>-0.23 (-0.59, 0.12)</td>
<td>0.85 (0.66-1.09)</td>
<td>1.08 (0.81-1.46)</td>
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<td>Home Ownership</td>
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<td>Rents vs. Owns Home</td>
<td>0.89 (0.64, 1.15)</td>
<td>1.86 (1.56-2.21)</td>
<td>-0.02 (-0.26, 0.22)</td>
<td>0.99 (0.84-1.17)</td>
<td>1.86 (1.56-2.21)</td>
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<td>Household Income</td>
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<td>$50,000-99,999/year vs. &lt;$50,000/year</td>
<td>-0.93 (-1.25, -0.61)</td>
<td>0.52 (0.42-0.65)</td>
<td>-0.34 (-0.62, -0.07)</td>
<td>0.79 (0.65-0.95)</td>
<td>0.52 (0.42-0.65)</td>
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<td>$100,000-149,999/year vs. &lt;$50,000/year</td>
<td>1.18 (0.89, 1.47)</td>
<td>2.26 (1.85-2.77)</td>
<td>0.38 (0.13, 0.64)</td>
<td>1.31 (1.09-1.56)</td>
<td>2.26 (1.85-2.77)</td>
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<td>$150,000+/year vs. &lt;$50,000/year</td>
<td>0.22 (-0.02, 0.46)</td>
<td>1.17 (0.99-1.37)</td>
<td>0.05 (-0.14, 0.25)</td>
<td>1.04 (0.91-1.19)</td>
<td>1.17 (0.99-1.37)</td>
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<tr>
<td>Prefers not to say vs. &lt;$50,000/year</td>
<td>0.24 (0.03, 0.45)</td>
<td>1.18 (1.02-1.36)</td>
<td>0.11 (-0.06, 0.28)</td>
<td>1.08 (0.96-1.21)</td>
<td>1.18 (1.02-1.36)</td>
</tr>
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<td>Collection Season</td>
<td>0.32</td>
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<tr>
<td>Spring vs. Fall</td>
<td>-0.18 (-0.49, 0.12)</td>
<td>0.88 (0.71-1.08)</td>
<td>-0.34 (-0.58, -0.10)</td>
<td>0.80 (0.67-0.93)</td>
<td>0.88 (0.71-1.08)</td>
</tr>
<tr>
<td>Summer vs. Fall</td>
<td>0.13 (-0.18, 0.45)</td>
<td>1.10 (0.88-1.36)</td>
<td>0.18 (-0.06, 0.43)</td>
<td>1.13 (0.96-1.34)</td>
<td>1.10 (0.88-1.36)</td>
</tr>
<tr>
<td>Winter vs. Fall</td>
<td>-0.21 (-0.52, 0.11)</td>
<td>0.87 (0.70-1.08)</td>
<td>-0.32 (-0.57, -0.07)</td>
<td>0.80 (0.67-0.95)</td>
<td>0.87 (0.70-1.08)</td>
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<tr>
<td>Predictor variables (n=19)</td>
<td>3HC Model Coefficients (95% CI)</td>
<td>Multiplicative Change in Concentration (95% CI)</td>
<td>R² (%) of Multivariable 3HC Model Coefficients (95% CI)*</td>
<td>Multiplicative Change in Concentration (95% CI)</td>
<td>Model R² (%)</td>
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<td>Study Centre</td>
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<td>40.89</td>
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<tr>
<td>Toronto vs. Edmonton</td>
<td>-0.50 (-0.83, -0.17)</td>
<td>0.71 (0.56-0.89)</td>
<td>-0.11 (-0.40, 0.17)</td>
<td>0.92 (0.76-1.12)</td>
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</tr>
<tr>
<td>Vancouver vs. Edmonton</td>
<td>-0.82 (-1.14, -0.51)</td>
<td>0.57 (0.45-0.70)</td>
<td>-0.59 (-0.86, -0.31)</td>
<td>0.67 (0.55-0.80)</td>
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<tr>
<td>Winnipeg vs. Edmonton</td>
<td>0.06 (-0.24, 0.37)</td>
<td>1.05 (0.84-1.29)</td>
<td>-0.15 (-0.40, 0.10)</td>
<td>0.90 (0.76-1.07)</td>
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<td>Paternal Education</td>
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<td>7.05</td>
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<tr>
<td>Some post-secondary vs. Highschool or less</td>
<td>-1.58 (-1.85, -1.31)</td>
<td>0.33 (0.28-0.40)</td>
<td>-0.45 (-0.70, -0.20)</td>
<td>0.73 (0.62-0.87)</td>
<td></td>
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<tr>
<td>Completed post-secondary vs. Highschool or less</td>
<td>0.23 (0.02, 0.47)</td>
<td>1.17 (0.99-1.39)</td>
<td>-0.11 (-0.31, 0.10)</td>
<td>0.93 (0.81-1.07)</td>
<td></td>
</tr>
<tr>
<td>Masters or PhD vs. Highschool or less</td>
<td>0.18 (-0.03, 0.40)</td>
<td>1.14 (0.98-1.32)</td>
<td>0.20 (0.25, 0.37)</td>
<td>1.15 (1.02-1.30)</td>
<td></td>
</tr>
</tbody>
</table>

Estimated change in log-transformed metabolite level by predictors in unadjusted regression models (95% confidence intervals), as well as the coinciding R² values are shown to the nearest second decimal place. Estimated change in log-transformed metabolite level by predictors in **adjusted** final regression models (95% confidence intervals), as well as the coinciding R² values are shown to the nearest second decimal place.
Appendix B.

Supplemental Information for Chapter 3: The relationship between infant urinary nicotine metabolites and childhood asthmatic disease; Exploring the role of breastfeeding and diet.

Appendix B figures and tables

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Figure B.1. Sample Selection Flow Chart

Of the 3,455 children in the CHILD Study, 2,607 had urine samples collected at 3 months of age. 2,607 urine samples are available, with 2,509 of these samples having detectable levels of our biomarkers available for analysis. We examined 2,570 urine samples taken at 3-4 months of age to measure cotinine and trans-3’-hydroxycotinine (3HC), and a combination of both. A subset of these who have complete data of relevant demographic, socioeconomic, exposure, diet, and health outcome data were selected as the current sample size. Of the final 1,432 subjects with metabolite data, 361 (25.2%) had a cotinine concentration below the LOD, and 162 (11.3%) had a 3HC concentration below the LOD that were imputed prior to analysis.
Figure B.2 Scatterplots of the association between maternal dietary nicotine intake during pregnancy and their infant’s urinary cotinine concentration.
# Table B.1  Calculation of Maternal Dietary Nicotine Intake during Pregnancy

<table>
<thead>
<tr>
<th>Vegetable</th>
<th>Average nicotine content</th>
<th>Average amount per portion; *CHILD variable</th>
<th>Calculations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peeled tomato sold in cans; tomatoes</td>
<td>3.9 µg kg⁻¹ ww⁶</td>
<td>130g per 125mL</td>
<td>3.9 ug/kg * 1000 ng/ug * kg/1000g = 3.9 ng/g</td>
</tr>
<tr>
<td></td>
<td>7.3 ng/g ww⁻¹</td>
<td>123g per raw tomato</td>
<td>3.9 ng/g * 123 g/portion = 479.7 ng/portions</td>
</tr>
<tr>
<td></td>
<td>Highest:</td>
<td>*Ffrq73 (Fresh tomatoes)</td>
<td>479.7 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td></td>
<td>4.1-4.3 ng/g CM,S&lt; - ripe, fresh</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tomato sauce with vegetables</td>
<td>6.2 µg kg⁻¹ ww⁶</td>
<td>130g per 125mL</td>
<td>(6.2+4.5)/2 = 5.35</td>
</tr>
<tr>
<td></td>
<td>52.0 ng/g⁻³ &lt;- pureed tomatoes (highest conc)</td>
<td>130g per 125mL</td>
<td>5.35 ng/g * 130 g/portion = 695.5 ng/portions</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>695.5 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td>Tomato sauce, with spices</td>
<td>4.5 µg kg⁻¹ ww⁶</td>
<td>130g per 125mL</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>*Ffrq48 (Spaghetti and other pasta with tomato and meat sauce)</td>
<td></td>
</tr>
<tr>
<td>Ketchup sold in glass or plastic</td>
<td>Avg 7.3 µg kg⁻¹ ww⁶</td>
<td>30mL (2 tbsp)</td>
<td>7.3 ng/g * 30 g/portion = 219 ng/portions</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*1ml ketchup=0.95g ketchup</td>
<td>219 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*Ffrq105 (ketchup)</td>
<td></td>
</tr>
<tr>
<td>French fries</td>
<td>9.2 µg kg⁻¹ ww⁶</td>
<td>48g per 20 strips</td>
<td>9.2 ng/g * 48 g/portion = 441.6 ng/portions</td>
</tr>
<tr>
<td>Vegetable</td>
<td>Average nicotine content</td>
<td>Average amount per portion; *CHILD variable</td>
<td>Calculations</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>--------------------------</td>
<td>---------------------------------------------</td>
<td>--------------------------------------------------</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*Ffrq92 (French fries, fried potatoes and hash browns)</td>
<td>441.6 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td>Baked potatoes, cooked and peeled</td>
<td>3.3-7.6 µg kg(^{-1}) ww(^{a})</td>
<td>156 per baked potato (173 with skin) *Ffrq93 (Potatoes - boiled, baked or mashed)</td>
<td>7 ng/g * 156 g/portion = 1092 ng/portions 1092 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td></td>
<td>15 ng/g ww(^{d}) (potato)</td>
<td>7 ng/g(^{S}) (potato)</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>7 ng/g(^{S}) (potato)</td>
<td>7 ng/g (potato)</td>
<td>N/A</td>
</tr>
<tr>
<td>Eggplant</td>
<td>2.9 µg kg(^{-1}) ww(^{a})</td>
<td>52g per 125mL</td>
<td>N/A</td>
</tr>
<tr>
<td></td>
<td>82g per 125mL (74g per ½ pepper raw)</td>
<td>*Ffrq75 (Green peppers and green chilies)</td>
<td>3.7 ng/g * 82 g/portion = 303.4 ng/portions 303.4 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td></td>
<td>82g per 125mL (74g per ½ pepper raw)</td>
<td>*Ffrq75 (Green peppers and green chilies)</td>
<td>3.7 ng/g * 82 g/portion = 303.4 ng/portions 303.4 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td>Green peppers</td>
<td>3.7 µg kg(^{-1}) ww(^{a})</td>
<td>82g per 125mL (74g per ½ pepper raw)</td>
<td>3.7 ng/g * 82 g/portion = 303.4 ng/portions 303.4 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td></td>
<td>(74g per ½ pepper raw)</td>
<td>*Ffrq75 (Green peppers and green chilies)</td>
<td>3.7 ng/g * 82 g/portion = 303.4 ng/portions 303.4 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td>Red peppers</td>
<td>5.9 µg kg(^{-1}) ww(^{a})</td>
<td>74g per 125mL (60g per ½ pepper raw) *Ffrq76 (Red peppers and red chilies)</td>
<td>5.9 ug/kg * 1000 ng/ug * kg/1000g = 5.9 ng/g 5.9 ng/g * 74 g/portion = 436.6 ng/portions 436.6 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td></td>
<td>(60g per ½ pepper raw)</td>
<td>*Ffrq76 (Red peppers and red chilies)</td>
<td>5.9 ug/kg * 1000 ng/ug * kg/1000g = 5.9 ng/g 5.9 ng/g * 74 g/portion = 436.6 ng/portions 436.6 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td>Vegetable</td>
<td>Average nicotine content</td>
<td>Average amount per portion; *CHILD variable</td>
<td>Calculations</td>
</tr>
<tr>
<td>---------------------------------</td>
<td>--------------------------</td>
<td>---------------------------------------------</td>
<td>------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Commercial black tea, brewed</td>
<td>4.0 µg L⁻¹ s = 4.0 ng/g</td>
<td>250mL per cup</td>
<td>Avg. 3.5 ng/g</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*Bfrq4 (Tea (all types))</td>
<td>3.5 ng/g * 250 g/portion = 875 ng/portions</td>
</tr>
<tr>
<td>Commercial green tea, brewed</td>
<td>2.02 ppm⁰⁶⁹</td>
<td>250mL per cup</td>
<td>875 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
<tr>
<td></td>
<td>1 ppm = 1000ng/mL</td>
<td>*1g water=1mL water</td>
<td></td>
</tr>
<tr>
<td>Cauliflower</td>
<td>3.8 ng/g⁵⑥ ww</td>
<td>66g per 125mL, 53g if raw</td>
<td>3.8 ng/g * 66g/portion = 250.8 ng/portion</td>
</tr>
<tr>
<td></td>
<td></td>
<td>*Ffrq79 (Cauliflower and Brussels sprouts)</td>
<td>250.8 ng/portion * portions/meal * frequency eaten/week = _ng intake/week</td>
</tr>
</tbody>
</table>

Information on the wet-weight (ww) nicotine content of vegetables and tea were sourced from 3 peer-reviewed articles. Portion sizes were based on content per portion, as determined by Health Canada (Health Canada, 2008). Raw codes for ffrq and bfrq variables were converted into values that represent average weekly number of portions of that particular food item.

- $X^3 = (\text{Siegmund, Leitner, & Pfannhauser, 1999})$
- $X^3 = (\text{Davis, Stiles, DeBethizy, & Reynolds, 1991})$
- $X^{SH} = (\text{Sheen, 1988})$


