TRICLOSAN: SOURCE ATTRIBUTION, URINARY METABOLITE LEVELS AND TEMPORAL VARIABILITY IN EXPOSURE AMONG PREGNANT WOMEN IN CANADA

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ABSTRACT

OBJECTIVE:

To measure urinary triclosan levels and their variability across pregnancy, and to identify sources of triclosan exposure among Canadian pregnant women.

METHODS:

Single spot and serial urine samples, as well as consumer product use information were collected across pregnancy and post-partum from 80 healthy pregnant women in Ottawa. Analyses included descriptives, linear mixed effects and parametric trend modeling, and surrogate category analysis.

RESULTS:

Triclosan was detected in 87% of maternal urine samples (LOD=3.0 μg/L). Triclosan concentrations varied by time of day of urine collection (p=0.0006), season of sampling (p=0.019), and parity (p=0.038). Triclosan was included in 4% of all personal care products used by participants; 89% of these triclosan products were varying brands of toothpaste and hand soaps.

CONCLUSION: This study provided the first data on temporal variability urinary triclosan levels, and on source attribution data in Canadian pregnant women. Results will assist with population-specific exposure assessment strategies.

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CHAPTER 1: INTRODUCTION TO THE STUDY

1.1 THE PROBLEM

Triclosan (TCS) is a current topic of interest due to concerns about potential adverse health effects and its widespread use. Research studies and media stories are focusing on this antibacterial agent due to the steady increase in the number of triclosan-containing consumer products over the past 20 years, including toothpaste, hand soap, deodorant and mascara to name a few (Alliance for the Prudent Use of Antibiotics, 2011; Sandborgh-Englund et al., 2006). A recent report published in 2012 by Environmental Defence labeled the bioaccumulation of triclosan in the environment as "toxic to the aquatic environment" (Environmental Defence 2012). The widespread use of triclosan has also been linked to occurrence of TCS antimicrobial resistance in dermal, intestinal, and environmental microorganisms (Yazdankhah et al. 2006). This begs the question as to whether or not there are further human health impacts due to triclosan exposure.

Nearly all of the research on potential health effects of triclosan use animal models (The Associated Press 2013); however, the results from these models are not always applicable to humans (FDA 2010). Current research on potential human toxicity of triclosan has demonstrated that it has low acute toxicity in humans who use the products as intended (Rodricks et al., 2010), and its rapid excretion through urine and feces creates a low chronic health risk from the current use of triclosan-containing products (NICNAS 2009). However, there are knowledge gaps on triclosan source attribution and current exposure levels among certain populations; especially among susceptible populations such as Canadian pregnant women. This information is critical for risk assessment of triclosan and to guide risk management options.

1.1.1 Triclosan knowledge gaps

Triclosan has been consistently detected in approximately 75% of participants in surveys conducted in the United States and Canada (Calafat et al. 2008; Environmental Defence 2012; Health Canada 2013). A recent Canadian population-based survey (Health Canada 2013b) as well as a smaller Canadian study (Environmental Defence 2012) reported detection of urinary triclosan levels among 88% and 72% of their participants, respectively; however, these surveys did not target a population of pregnant women.

At the time of creating a study analysis plan, there were no studies published on the temporal variability of triclosan. However, methods for assessing the temporal variability as well as personal care product use for phthalates had been developed. Similar to triclosan, phthalates are ubiquitous in the environment, primarily excreted in urine, do not lead to bioaccumulation in humans, and have short half-lives of less than 24 hours in urine or feces (Agency for Toxic Substances and Disease Registry 2002).

Phthalates are a family of chemicals added to a number of industrial and consumer products in order to increase product flexibility. Some products containing phthalates include vinyl plastics and flooring, hairsprays, cosmetics and insect repellants (Health Canada 2011b; Wolff et al. 2007), which can release phthalates into the environment.

Urinary phthalate levels have been positively associated with personal care product use (Hauser et al, 2004). In addition, other factors such as diet, activity patterns and other environmental and biological factors may influence exposure levels (Hauser et al. 2004). To date, only one research study provides information on source attribution to triclosan. This study was conducted in a sample of Puerto Rican pregnant women between 2010 and 2012. Positive associations were identified between triclosan concentrations and the use of certain personal care products (Meeker et al., 2013). This research study, among others, has

highlighted the importance of conducting additional research to support existing data on triclosan levels and source attribution in human populations (Calafat et al. 2008; Meeker et al. 2013). Health Canada has used U.S. population data to provide the most accurate estimates of total triclosan exposure of the general population (Health Canada and Environment Canada 2012). This highlights the need for Canadian source attribution data.

Significant costs and participant burden arise when collecting and analyzing serial urine samples. As a result, epidemiologic studies or large population surveys commonly measure an individual's exposure to a chemical through a single spot urine sample; however, it is unclear whether using a single spot urine sample to measure triclosan is a representative indicator of an individual's exposure over a period of time. Following exposure to triclosan, the urinary triclosan concentration fluctuates; it degrades by 50 percent in approximately 11 hours (Sandborgh-Englund et al. 2006). Limited data exist on this matter, especially in pregnant women.

To date, there is a paucity of Canadian data on current urinary triclosan levels in pregnant women, as well as the sources of exposure, temporal variability, and reliability of a single spot urine sample in a pregnant population.

1.2 SIGNIFICANCE

The potential health effects, if any, of triclosan (TCS) exposure on humans are largely unknown, but based on experimental toxicology studies, are expected to be minimal. Clearly having information on urinary triclosan levels in a Canadian population is important for consumers and for those working in areas of risk assessment and risk management in order to properly assess the exposure levels and triclosan product uses in order to characterize risk, if any, that may be associated with the chemical. This study contributes to

the ongoing research by providing the first Canadian data in a subpopulation of pregnant women. This population is of specific interest due to the fragility of the developing fetus and its increased vulnerability relative to the susceptibility of adults to endocrine modulation effects (Casas et al. 2011; WHO 2012).

This thesis will produce the first report of Canadian data on maternal urinary triclosan levels and on the temporal variability of triclosan through assessment of TCS concentrations within a 24-hour time period. This information will provide insight into potential exposure misclassification when only a spot urine sample is collected and recommendations for when and what additional data to gather, if only a spot urine sample is collected.

Identification of which sources of exposure are most correlated with urinary TCS levels can further direct or enhance exposure controls and risk management procedures.

1.3 THE PURPOSE STATEMENT

This thesis focuses on triclosan data collected from the P4 Study, a longitudinal observational study examining exposure of Canadian pregnant women to triclosan, phthalates, bisphenol A, naphthalene, cotinine and triclocarban.

Following data entry, product categorization, variable creation, as well as dataset merging and cleaning, analysis and interpretation of the P4 Study triclosan data will be performed.

The purpose of this thesis is to correlate urinary maternal triclosan levels with personal care product use throughout pregnancy among Canadian pregnant women, while controlling for potential confounding variables. In addition, this study will contribute to the ongoing research on the ability of a single spot sample taken at varying time points throughout pregnancy and postpartum to accurately predict individual exposure levels.

Results from this study can assist with population-specific exposure assessment strategies in Canadian populations and can contribute to ongoing assessment studies worldwide. The methodology for analysis of triclosan data developed in this thesis will then be used for data analysis of the remaining chemicals of interest in the P4 Study.

CHAPTER 2: THEORETICAL FRAMEWORK, HYPOTHESES AND OBJECTIVES

2.1 THEORETICAL FRAMEWORK

In 1983, a risk assessment paradigm was established in The United States Red Book to provide information on concepts and conduct of a systematic risk-assessment process. Today, this paradigm is still a core component of newly developed frameworks that are advancing the field of risk assessment through further emphasis on the options available to reduce exposure and evaluate the hazards (Abt et al., 2010) . The Red Book framework has been referenced by expert committees, regulatory agencies and public health institutions in their decision-making processes (National Academy of Sciences 2008). The original risk assessment paradigm, as listed in the Red Book will be the basis of presentation of published literature in this area, due to its pertinence to chemical risk assessment. Figure 1 represents the theoretical framework for this study.

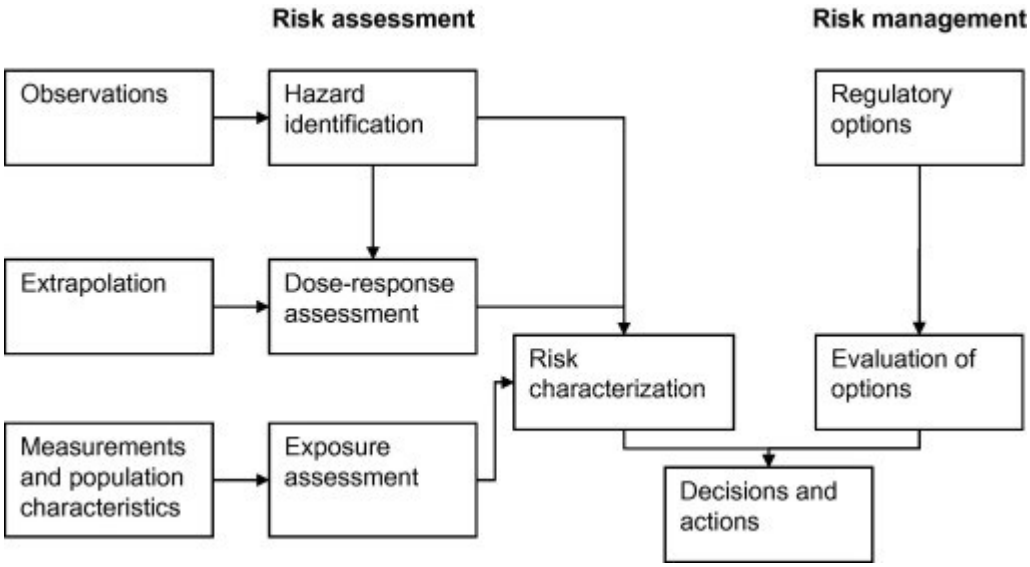


Figure 1. The Red Book risk assessment process (Pohjola et al. 2012).

2.2 DEFINITIONS

General concepts for the purpose of this thesis are summarized as follows:

Temporal variability = within-individual variation of urinary triclosan concentrations across time.

Source attribution = various products through which an individual is exposed to triclosan.

Personal care products = substances or mixtures of substances which are generally recognized by the public for use in daily cleansing or grooming (Environment Canada 2012).

Personal care product category = a combination of individual personal care products with similar characteristics. Selected product categories for this thesis include: cosmetics, hair care, oral care, deodorant/antiperspirant, hand soaps/sanitizers, lotions/creams, skin care, medication/vitamins, household cleaning

products, and other products.

The following variables are presented in the statistical analyses of this study:

Dependent variable: urinary triclosan concentrations in pregnant women (spot urine and serial urine samples collected over a 24-hour period).

Independent variable: personal care product use summary score (sum of total number of exposures to an individual personal care product containing triclosan).

Covariates: maternal age, education, marital status, combined household income, country of birth, time of day of urine void, season of sampling, season of conception, parity, total urine volume, study visit, and time since last urine void.

2.3 OBJECTIVES

The objectives of this study in our population of Canadian pregnant women are:

Objective 1. To highlight the main personal care product sources of exposure to triclosan and their association with urinary triclosan concentrations.

Objective 2. To identify covariates which are statistically significant predictors of urinary triclosan levels.

Objective 3. To measure current triclosan exposure levels through urine biomonitoring.

Objective 3a. To measure inter-subject and within-subject variability of urinary triclosan levels:

- Within a week-end day and a week-day
- At various stages of pregnancy
- Over a 24-hour period

Objective 3b. To evaluate the ability of a single spot urine sample to correctly predict an individual's level of exposure to triclosan.

Objective 4. To determine the pattern of urinary triclosan concentrations in a 48-hour time period following triclosan exposure.

2.4 HYPOTHESES

It is hypothesized that:

Hypothesis 1. Pregnant women will primarily be exposed to triclosan through cosmetics, soaps, and toothpaste.

Hypothesis 2. Triclosan levels will be predicted by time of day of sample collection.

Hypothesis 3. Canadian triclosan exposure levels measured through urine voids among pregnant women will be similar to those found in comparable studies. Specifically, triclosan will be detected in 60-83% of maternal urine samples.

Hypothesis 3a. Inter-subject variability will be attributed primarily to differences in the timing of exposure to triclosan-containing products.

Hypothesis 3b. A single spot sample will prove to be a reliable indicator of an individual's average exposure to triclosan-containing products.

Hypothesis 4. Triclosan levels will experience a sharp increase following exposure to a triclosan-containing product, followed by a constant decline, with a half-life of approximately 11 hours.

CHAPTER 3: REVIEW OF THE LITERATURE

The literature review section will first provide a basic introduction to triclosan, identify products in which triclosan is present, and outline its impacts on the environment, animals, and humans. A brief history of biomonitoring will also be presented, addressing the advantages and challenges of different biomonitoring matrices, as well as additional challenges that arise in populations of pregnant women.

Following this, examination of evidence relating to triclosan biomonitoring studies in various populations will be conducted, including studies measuring levels following triclosan exposure. Together, this information will support the risk characterization of triclosan.

Deficiencies and limitations in the existing literature will define the purpose of the present study.

3.1 RISK ASSESSMENT

3.1.1 Hazard identification

Chemicals are ubiquitous in today's environment. Human exposure to these chemicals is unavoidable. To assist with quantification of exposure, chemicals are classified according to their persistency. Persistency is defined as "the residence time of a chemical species in a specifically defined compartment of the environment (Greenhalgh et al. 1980)", with respect to the chemical and physical properties of the agent. Further persistency is described through "the dispersion of the chemical agent from its primary compartment to a new location (US Environmental Protection Agency 2004)". Estimation of persistency depends on three basic processes: how the chemicals are released, how they move in their

environment and their tendency to degrade within their compartment (US Environmental Protection Agency 2004).

Persistency can be sub-divided into two categories: persistent or non-persistent, according to the time required for the compound to disperse. The US Environmental Protection Agency lists compounds that never degrade or that require a very long period of time to do so as persistent. On the other hand, non-persistent chemicals are those that degrade over a very short period of time (US Environmental Protection Agency 2004).

The degradation time of a chemical determines its half-life. Half-life is defined as "the period it takes for the concentration of a substance to be reduced by half, by transformation, in a medium (Canadian Environmental Protection Act 2000)". There exists no single threshold separating persistent versus non-persistent compounds, most importantly because chemicals degrade at different rates according to the environmental conditions of specific media. Specifically, factors such as temperature, types of microbes present in the environment, and concentrations of the compounds can all influence the chemical half-life (Verschueren 2001).

Triclosan is classified as a non-persistent chemical in air, water, soil and sediment because its half-life does not meet any of the criteria for each of the above medias, as identified in the *Persistence and Bioaccumulation Regulations* of the *Canadian Environmental Protection Act, 1999* or the Toxic Substances Management Policy (Health Canada and Environment Canada 2012). Persistent chemicals must meet at least one of the following characteristics (Canadian Environmental Protection Act 2000):

"(*a*) in air,

(i) its half-life is equal to or greater than 2 days, or

(ii) it is subject to atmospheric transport from its source to a remote area;

(*b*) in water, its half-life is equal to or greater than 182 days;

(*c*) in sediments, its half-life is equal to or greater than 365 days; or

(*d*) in soil, its half-life is equal to or greater than 182 days."

3.1.2 Characteristics and sources of exposure of triclosan

Triclosan is a broad-spectrum anti-microbial agent that was initially developed in Basel, Switzerland, and has been used to control the spread of bacteria since as early as the 1960s (Fang et al., 2010; Jones et al., 2000). This agent is also marketed under the following brand names: Microban, Amicor, Aquasept, Bactonix, DP 300, Irgasan, Monolith, Sanitized, Sapoderm, Ster-Zac and Ultra-Fresh (Environmental Defence 2012).

Triclosan is commonly added to over 1500 personal-care products including toothpastes, mouthwashes, soaps, deodorants, and cosmetics (Government of Canada 2013). In addition, it exists in many medical devices and household items such as plastic kitchen utensils, toys, (Clayton et al., 2011; Fang et al., 2010; Kim et al., 2011; Philippat et al., 2012) and even smartphone cases (Environmental Defence, 2012).

Research on wastewater and surface water monitoring in the United States has concluded that due to the abundance of consumer products containing triclosan, it is one of the most frequently detected compounds and in highest concentrations in wastewater, due to disposal in residential drains (Reiss et al., 2002). Washing hands and dishes, as well as bathing and brushing teeth with triclosan-containing personal care products are a select few of the activities that can result in accumulation of triclosan in waterways through product disposal. Humans generally have low exposure to triclosan from drinking water due to the water treatment process. Water treatment plants have a triclosan removal efficiency rate of approximately 95% (Samsoe-Petersen et al., 2003). The accumulation of triclosan in waterways results in chronic exposure of aquatic organisms such as fish, frogs and algae

(Environmental Defence 2012). Consequently, concentrations found in fish are much higher than those found in water (Balmer et al., 2004; Samsoe-Petersen et al., 2003). Triclosan accumulates in these media (Bennett et al., 2009; Fair et al., 2009). Upon consumption of fish, humans may also be exposed to triclosan, albeit in negligible amounts.

Although TCS lacks the ability to persist in aerobic conditions, its continual input to surface water through wastewater treatment plants makes exposure to this chemical agent ubiquitous (Canadian Environmental Protection Act 2000).

3.1.3 The impact of triclosan on the environment, animals, and humans

3.1.3.1 Triclosan in the environment

Upon combined exposure with chlorine and UV radiation, triclosan undergoes photochemical degradation and produces 2 dioxins (2,8-dichlorodibenzo-*p*-dioxin (2,8-DCDD) and 2,4-dichlorophenol (2,4-DCP) (Alliance for the Prudent Use of Antibiotics 2011). Dioxins are highly toxic, persistent environmental pollutants that can affect reproduction, development, immunity, and hormones; they can also be carcinogenic (World Health Organization 2010). There are 210 environmental dioxins; however, only 17 are of public health concern (Van den Berg et al. 2006). It has been concluded that the two formations resulting from triclosan degradation are not of public health concern (Latch et al. 2005). This is important to confirm that human exposure to triclosan through these dioxins is a negligible source.

3.1.3.2 Animal models of triclosan toxicity

Conclusions drawn from animal research studies have identified mixed androgenic and thyroid effects due to triclosan exposure. Triclosan mimics the thyroid hormone, thereby restricting the functionality of endogenous hormones and inhibiting the metabolism of the thyroid hormone. This thyroid hormone-associated disruption at low levels of exposure (0.03

mg/L) has been linked to disruption of gene expression in tadpoles (Veldhoen et al. 2006) and reduced sperm production in male rats (Kumar et al. 2009). Other research has shown that TCS hinders estrogen sulfotransferase, an important enzyme in the metabolization and transportation of the hormone to the fetus in sheep placenta (James et al. 2010). In addition, triclosan has been found to impact testosterone binding in rats (Gee et al., 2008). Chronic oncogenicity studies have identified tumors in mice, rats, and hamsters; however, the development of similar tumors in humans is not plausible due to differences in TCS metabolite generation and excretion as well as modes of actions in the animals that are not relevant to humans (FDA 2010; Rodricks et al. 2010).

On the contrary, further research has shown that triclosan has no effect on androgenic activity in rats (Zorrilla et al., 2009) or in frogs (Matsumura et al., 2005). As well, no reproductive effects were found on Japanese Medaka fish after 21 days of triclosan exposure (Ishibashi et al., 2004).

3.1.3.3 Impact of triclosan on human health

Antimicrobial agents can be bacteriostatic or bactericidal. Bacteriostatic agents prevent the growth of microorganisms without resulting in microorganism death, whereas bactericidal agents kill microorganisms directly. Triclosan is bacteriostatic at low concentrations and bactericidal at higher concentrations (Alliance for the Prudent Use of Antibiotics 2011). It has an effect on many types of Gram-positive and Gram-negative non-sporulating bacteria, as well as on some fungi and parasites (McLeod et al. 2001; Schweizer 2001). It does not have an effect on *Pseudomonas aeruginosa* or on *Clostridium difficile* (Alliance for the Prudent Use of Antibiotics 2011).

The efficacy of the anti-microbial activity of triclosan-containing products varies. The efficacy of triclosan-containing soaps was examined using bacterial counts and was found to not be statistically significant from the bacterial counts in regular soaps (Aiello et al., 2007; Tan et al., 2002). Similarly, there was no distinct evidence of efficacy of triclosan as an anti-microbial agent in plastics (Fang et al. 2010). Contrarily, in Colgate Total® toothpaste, TCS was found to successfully prevent gingivitis (FDA 2010). However, two studies examining exposure to triclosan through toothpaste use showed no significant differences in plasma thyroid levels following short-term or long-term use of the toothpaste (Cullinan et al., 2010; Allmyr et al., 2009).

Although triclosan has been linked to antimicrobial resistance (Yazdankhah et al., 2006), the majority of research studies show no association between triclosan and bacterial resistance (Aiello et al., 2007; Randall et al., 2004; Russell 2004; Suller & Russell, 2000; Russell 2000; Beier et al., 2008).

Triclosan can lead to endocrine disruption (Allmyr et al., 2009; Kim et al., 2011). An endocrine disrupting chemical (EDC) alters hormone signaling and can have potential effects on hormone homeostasis (2009) and on transcriptional activity induced by testosterone (Chen et al. 2007), on metabolism, as well as on the development of reproductive and nervous systems (Dann and Hontela, 2011; Dodson et al., 2012). To date, only one epidemiologic study has examined the potential effects of TCS on thyroid status. An analysis of the US National Health and Nutrition Examination Survey (NHANES) data (a cross-sectional study) from 2007-2008 has reported a positive association between triclosan and total plasma triiodothyronine (T3) concentrations in adolescents; T3 levels were unaffected in adults (Koeppe et al. 2013; Meeker et al. 2013). Triiodothyronine is a thyroid hormone responsible for fetal and child growth and neurodevelopment, as well as regulation of

metabolism, reproductive, and cardiovascular systems (Dussault and Ruel, 1987; Stathatos 2012). Pregnant women are more susceptible to the effects of increased thyroid levels; symptoms of increased T3 levels include accelerated central nervous system functioning such as increased heart rate, metabolism, and anxiety. Anti-thyroid medication can cross the placenta. This can lead to underdevelopment of the fetus due to low thyroid levels (Springhouse Corporation, 1984).

In addition to the above effects, contact dermatitis and skin irritation can occur following contact with triclosan (Robertshaw and Leppard, 2007). Also, photo-allergic contact dermatitis (PACD), characterized by a more severe rash, can also result following dermal exposure and subsequent ultraviolet radiation (Schena et al., 2008).

3.1.4 Exposure Assessment

Through dermal and oral routes, personal care products are the primary source of exposure to triclosan. Exposure pathways such as fish consumption and contact with untreated wastewater are negligible sources of triclosan exposure when compared to dermal or oral routes. Triclosan is absorbed in the gastrointestinal tract and across the skin (Dayan 2007); however, to date there is no evidence of bioaccumulation from human oral or dermal exposures to triclosan (SCCP (Scientific Committee on Consumer Products) 2009). For this reason, exposure to personal care products is the focus of this thesis. In addition to the exposure pathway, knowledge of the concentration, duration, frequency, and timing of exposure, as well as the chemical identity, source, and medium of transport are essential for the basis of risk assessment of environmental chemicals.

Indirect or surrogate methods of exposure data collection such as questionnaires, diaries and interviews are commonly used to measure activity patterns among the population. Although practical, these collection methods are self-reported and introduce a high amount

of uncertainty into the calculation of exposure estimates due to their subjectivity (Meeker et al. 2013; Prince et al. 2008; Shephard 2003). Direct methods such as measurement of a person's environmental exposure through for example, the use of an air monitor provide objective results; however, these methods tend to be expensive, and generally do not provide any detailed information pertaining to sources of entry of the chemical into the human body nor on confounding exposures. In addition, measures of the external environment often assess only one route of exposure – for example, inhalation and may be a less precise indicator of internal dose. Because of the strengths and drawbacks of both indirect and direct data collection methods, these methods should be combined to obtain more precise measures of exposure to environmental chemicals.

3.2 BIOMONITORING

Historically, exposure was measured by the degree of contact with a substance. Resulting information was inaccurate; however, the concept of absorbed dose was discovered. The absorbed dose is the amount of a compound that crosses the body's boundaries (Sexton et al. 2004). Today, the absorbed dose is measured through biomonitoring.

Biomonitoring is "the measurement of a chemical, the products it makes after it has broken down, or the products that might result from interactions in the body" (Health Canada 2013a). Biomonitoring data establishes baseline exposure levels, allows for comparison of exposure among populations, as well as supports future research on potential effects of environmental chemicals on human health (Health Canada 2013a). The direct measurements of chemicals or their metabolites in different biological matrices are referred to as biomarkers of exposure (California Department of Public Health 2013). Some collection

matrices include urine, blood, saliva, semen, hair, meconium, sputum, fingernails, lung tissue, bone marrow, adipose tissue, and blood vessels.

Biomonitoring data provides sound evidence of both exposure and uptake. These results can provide information on which subpopulations may be more vulnerable than others to exposure of a specific compound. The US National Health and Nutrition Examination Survey (NHANES) has demonstrated the value of biomonitoring. Between 1971 and 1975, the first cycle of this survey (NHANES I) collected a variety of health data from different populations. A plethora of research on exposure to environmental chemicals has since emerged and is used by the public, scientists, and other health professionals to perform exposure and risk assessments. The Fourth National Report on Human Exposure to Environmental Chemicals was published in 2009 (US Department of Health and Human Services 2013). In Canada, a similar population-based survey, the Canadian Health Measures Survey, is an ongoing biomonitoring and health examination survey that has now produced its second biomonitoring report (Health Canada, 2013b).

Biomonitoring has many advantages including accounting for all routes of exposure (dermal, oral, inhalation), as well as accurately representing repeated contact with a chemical (Arnold et al. 2013). Biomonitoring techniques today are used to precisely measure levels of environmental chemicals in human fluids and tissues.

3.2.1 Sampling methods: advantages and challenges

One of the most common biomonitoring matrices is blood, because of its contact with organs and tissues where chemicals are stored. Disadvantages of blood as a matrix, however, are that its collection is rather invasive (Esteban and Castaño, 2009) and that in pregnant populations, measurements of chemical concentrations in blood are influenced by plasma volume expansion (PVE). PVE occurs throughout pregnancy to provide for the circulatory

needs of the uterus, breasts, skin, kidneys, and placenta. The average volume expansion is 45%, although variation exists from minimum to a 2-fold increase. As a result, serum proteins are altered (Faupel-Badger et al. 2007). Plasma volume expansion is difficult to measure in population studies. In addition, concentrations of non-persistent chemicals may be significantly lower and of shorter duration in blood than in other matrices such as urine (Needham et al. 2008).

Another common biomonitoring matrix is urine. This is the predominant route of excretion for triclosan; urinary triclosan excretion proportions vary from 57 to 87% in United States literature (Rodricks et al. 2010). The urinary excretion half-life of TCS is approximately 11 hours (Sandborgh-Englund et al. 2006). Triclosan can be excreted in its free form, or it can undergo the chemical process of conjugation to increase its stability, which results in the excretion of conjugated forms of triclosan, specifically glucuronide and sulfate conjugates (Wang et al. 2004). The majority of triclosan is excreted through the urine within 24 hours (Fang et al. 2010) in its conjugated forms. As a result, urine void samples represent an accurate biomonitoring tool to measure TCS exposure (Calafat et al. 2008; Sexton et al. 2004). Other reasons that urine sample collections are excellent biomarkers of exposure include the ease of sample collection in addition to the fact that urinary metabolites are readily detectable for up to several days after exposure (Barr et al. 2006).

Although common and efficient, urine biomonitoring does present its own challenges. Serial urine samples over a 24-hour period raise the possibility of non-adherence by participants. Biomarker measurements may be affected by when during the day urine is collected, as well as the variability in the volume and concentration of the urine samples (Barr et al. 2006). As a result, the association between exposure and outcome may be misrepresented (Arbuckle 2010).

The collection of one single spot urine sample to represent the pregnancy period, as was performed in several studies of triclosan urinary concentration levels, does not allow for determination of the temporal variability of triclosan across pregnancy (Casas et al. 2011; Philippat et al. 2012; Wolff et al. 2008; Woodruff et al. 2011). An important question to ask is whether a single sample collected in the third trimester provides an accurate representation of exposure to a chemical that generally has a short half-life (Wolff et al. 2008), but may have a slightly different half-life due to metabolism differences in pregnant women as compared to non-pregnant women (Philippat et al. 2012).

With respect to other biomonitoring matrices, unique challenges present themselves in pregnant populations when correlating maternal exposure levels with fetal and infant exposure (Arbuckle 2010). In terms of breast milk collection, not all women breastfeed and providing extra milk for sampling can prove to be challenging for some women. In these cases, a breast pump is often used, which can lead to potential contamination of the sample from the collection materials. Lastly, standardizing the collection of breast milk sample is very difficult. Samples can be provided at any time of the day, all from one breast or through a serial collection from both breasts, and can be a combination of hind and fore milk (higher fat content versus lower fat content and available more at the end of feeding versus available at the beginning, respectively). When collecting cord blood, contamination may occur from collection materials. There may also be a competition for the sample and the delivery of a baby can be a hectic and unscheduled event. As a result, the collection of cord blood may not be a priority for the staff. Another matrix of interest is meconium, a tar-like substance that forms *in utero* around the thirteenth week of gestation and accumulates thereafter. Although collection of meconium is non-invasive and may provide a longer, cumulative record of

exposure to various environmental chemicals than urine or cord blood, its equivalency to other matrices is still unknown.

Additional factors should be taken into consideration when dealing with subpopulations such as pregnant women. There are critical time periods of exposure or susceptibility, and pregnant women are not a general healthy population. Pregnancies can be terminated for various reasons, health problems may present themselves, and there may be anxiety in providing the biospecimens for measurement of environmental chemicals. Pregnant women are routinely asked to provide maternal blood and urine as part of care; these can be used as surrogates for fetal exposure (Arbuckle 2010).

Measuring biomarkers of exposure can advance the field of research if they are true measurements of the individual's exposure for the relevant time period of interest (Arbuckle 2010). The wide number of biomonitoring matrices and the differences in collection methods between women raise challenges with respect to biomonitoring of pregnant women and infants.

3.2.2 Half-life

The selection of the biological matrix to measure exposure will depend on a number of factors, including the nature of the chemical of interest. Varying chemical and physical properties of environmental chemicals, along with the elimination half-life of each chemical can provide reason for measurement in one biological specimen over another. The half-life of a compound highly influences the selection of an appropriate biomonitoring matrix. Compounds with longer half-lives (months or years) have longer body biological residence times. These compounds can be sequestered in fatty acids or in bones and are therefore metabolized more slowly than others. Contrarily, compounds with short half-lives (hours or

days), such as triclosan, are quickly metabolized in non-fatty tissues and are often excreted in the urine (Sexton et al. 2004).

3.2.3 Urinary measurement of specific-gravity and creatinine

To properly interpret the urinary concentration of a compound, it is important to account for the hydration status of the individual (Haddow et al. 1994; Miller et al. 2004). Two common measures of hydration status are creatinine (CR) and specific gravity (SG). Creatinine is a muscle activity by-product that is excreted from the bloodstream by the renal system. Specific gravity is a measure of urine turbidity (Adibi et al. 2008). More specifically, it is a ratio of the density of the urine specimen to the density of water. Although many national studies including the US NHANES study report creatinine-adjusted urinary biomonitoring data (as well as the unadjusted data) (Calafat et al. 2008), adjusting for creatinine may introduce biases in either direction in the actual exposure dose. There has been substantial discussion among investigators as to the appropriateness of creatinine adjustment of single spot urine samples (Barr et al. 2005; Lee and Arbuckle, 2009). Intra-day variation exists in creatinine measurements due to a combination of both internal and external factors such as sex, age, health, diet, alcohol and incomplete voiding. As a result, other means of adjustment for urine dilution may be more accurate (Boeniger et al. 1993). Specific gravity has been identified as a useful alternative to creatinine in adjustment of urinary concentration levels (Berlin et al. 1985; Haddow et al. 1994; Miller et al. 2004), especially for women late in pregnancy (Adibi et al. 2008). Despite its sensitivity to changes in temperature resulting in an increase in inter and intra-subject variability (Miller et al. 2004), specific gravity measurement is rapid and inexpensive (Haddow et al. 1994) which makes it easy to use. Modern clinical refractometers to measure urine specific gravity include automatic temperature compensation.

3.2.4 Single versus serial urine sample collection

Valid and reliable measures of exposure are important in order to assess the toxicity of triclosan in humans. For some chemicals, several studies have shown that the between-subject variability is larger than within-subject variability, and as a result, a single urine sample may be representative of exposure (Hoppin et al. 2002; Mahalingaiah et al. 2008). For other environmental chemicals, moderate within-subject variation has been reported. This suggests that multiple urine samples are required to provide a reliable measure of an individual's exposure over a specified time period (Fromme et al. 2007; Hauser et al. 2004).

An intraclass correlation coefficient (ICC) is calculated to quantitatively measure the ability of biomarkers to properly measure exposure to a chemical. An ICC of 1.0 indicates perfect reproducibility, while an ICC of 0 indicates no reproducibility. A classification method used by Rosner in biomonitoring research is indicated in Table 1 (Rosner 2006).

Table 1. Interpretation of the intraclass correlation coefficient (ICC).

ICC	Reproducibility
<0.4	Poor
0.4 to 0.75	Fair to good
>0.75	Excellent

Due to the expense of laboratory analyses of biospecimens for environmental chemicals and the burden of multiple urine collections on participants, the collection of a single urine void from each individual in a large study or survey is common practice; however, the reliability of the measure can be questioned if the chemical has a short half-life

(such as triclosan) and if the potential for moderate within-subject variation exists. To date, only two studies have measured the temporal variability in exposure to triclosan. The first study (Teitelbaum et al. 2008) collected data from June to October 2004 in a sample of 35 children aged 6 to 10 years from New York. The children provided six spot urine samples within a six month time period. An intraclass correlation coefficient of 0.35 indicated poor reproducibility among samples collected at different time points (Rosner 2006). The second study (Meeker et al. 2013) collected data from 2010 to 2012 in a sample of 105 pregnant women from Northern Puerto Rico. Spot urine samples were only collected three times throughout pregnancy, for financial and logistical reasons. An intraclass correlation coefficient of 0.47 indicated a moderately consistent exposure to sources over time.

It is recommended that separate temporal variability studies should be conducted in pregnant women to develop population- and chemical-specific exposure assessment strategies (Hauser et al. 2004). To date, no studies have measured temporal variability in exposure to triclosan in a Canadian population, nor within a 24-hour period.

3.3 URINARY TRICLOSAN BIOMONITORING STUDIES

3.3.1 International studies measuring urinary triclosan in non-pregnant populations

Triclosan has previously been measured in several studies in the United States (Calafat et al. 2008; Clayton et al. 2011; Teitelbaum et al. 2008), as well as in Chinese and Korean populations (Kim et al. 2011; Li et al. 2011). Triclosan was detected in 93% of urine samples in both the Chinese and Korean populations, with limits of detection of 0.5µg/L and 0.05µg/L, respectively. The limits of detection in the United States studies were higher, ranging from 2.27 to 2.3µg/L. The detection rate of triclosan in these studies ranged from 70 to 75% (Calafat et al. 2008; Teitelbaum et al. 2008); however, these cannot be directly

compared to the Chinese and Korean studies because of the differences in the limits of detection.

Li and colleagues (2011) measured triclosan levels in a population (n=287) aged 3-24 years of age in China. They found a decreasing tendency in urinary triclosan levels with age. Contrarily, an increasing tendency in urinary triclosan levels with age was noted in a larger study by Kim and colleagues (2011), where triclosan levels were measured in a Korean adult population aged 18-69 years, as well as in a general United States population six years of age or older (Calafat et al. 2008).

Higher triclosan levels have also been found to be associated with higher income levels in the United States general population based on data from the National Health and Nutrition Examination Survey (Calafat et al. 2008; Clayton et al. 2011).

The cross-sectional nature and single spot urine sampling of these studies do not allow for the measurement of temporal variability of triclosan. Another limitation includes the generalizability of the results given the country of the population of interest. Specifically, in the study by Teitelbaum and colleagues (2008) in children of New York aged 6-10 years of age, only black and Hispanic children were included in the study, so they were not representative of the entire United States population of children.

A 2006 study (Sandborgh-Englund et al. 2006) examined the pharmacokinetics of triclosan following oral ingestion in humans. They found that within 4 days following oral exposure to triclosan, 24 to 83% of the dose was excreted. The median urinary half-life was found to be 11 hours (range 7-17 hours). This study described the pattern of plasma triclosan concentrations to increase rapidly with a maximum between 1 and 3 hours, and then slowly declined with a half-life of 21 hours. To date, models describing the pattern of urinary

triclosan concentration in a short time period or the rate of urinary triclosan concentration elimination have not yet been developed.

3.3.2 Triclosan in the Canadian population

Prior to May of 2012, no Canadian data on triclosan had been published. Since then, two separate reports have been published. The first report, published in May of 2012 (but not in the peer-reviewed literature), included descriptive statistics on urinary triclosan concentration measurements for 8 Canadians from the general population (Environmental Defence 2012). Detectable levels of triclosan were measured in 7 of these 8 participants (88%). Average urinary concentrations in this population were similar to those reported in the NHANES US population surveys (US Department of Health and Human Services 2013). Despite being the first study to provide triclosan data on a Canadian population, this was a survey of a very small and select population. In April of 2013, Health Canada published the "Second Report on Human Biomonitoring of Environmental Chemicals in Canada". This provided the results of the population-based Canadian Health Measures Survey (CHMS) Cycle II, which collected urine samples from 6,400 Canadians aged 3 to 79 years at 18 sites across Canada, from 2009-2011 (Health Canada 2013b). Detectable levels of triclosan were measured in 72% of the population, indicating again that exposure to triclosan is widespread. Other than the Environmental Defence and CHMS Cycle II data results, no other published Canadian data on triclosan exist at this time.

3.3.3 Triclosan in pregnant populations

Urinary triclosan concentrations have been measured in pregnant populations in the United States, Spain, France, and Puerto Rico (Casas et al. 2011; Meeker et al. 2013; Philippat et al. 2012; Wolff et al. 2008; Woodruff et al. 2011). Despite the relatively consistent limits of detection in these studies (levels varied from 2.3 to 2.7ng/mL), there was

high variability in the rates of detection among these studies. Triclosan was detected in urines of only 60% of the Spanish pregnant women in a 2011 study (Casas et al. 2011). In contrast, the detection rate was as high as 89% in a study by Meeker and colleagues (2013) of 105 Puerto Rican pregnant women from the Puerto Rico Test Site for Exploring Contamination Threats (PROTECT) study; however, differences in the proportion of the population using more of certain types of products may account for differences among studies (Woodruff et al. 2011).

The most recently published study on triclosan in Puerto Rican pregnant women measured the temporal variability of triclosan across pregnancy through serial urine sample collection, in addition to collecting information on distributions and predictors of urinary triclosan concentrations and self-reported product use information (Meeker et al. 2013). Consistent with results of general population studies (Calafat et al. 2008; Kim et al. 2011), the highest levels of urinary triclosan concentrations were present in the highest age category. Urinary triclosan levels in this population did not vary across pregnancy, as was determined by the similar measurements among study visits (Meeker et al. 2013).

Canadian data on triclosan in pregnant women, a population susceptible to increased thyroid levels (Springhouse, 1984) and endocrine modulation effects (Woodruff et al., 2008), have yet to be reported. This thesis project will produce the first Canadian data on the exposure levels and temporal variability in such a population.

A summary of studies measuring urinary triclosan concentrations in humans is detailed in Table 2.

Table 2. Summary of urinary triclosan studies in humans.

Author/Year	Objective	Population	Results	Limitations
Calafat et al., 2008	Exposure to triclosan in a representative sample	US general population (n=1288) ≥ 6 years of age 2003-2004 NHANES data	74.6% detection Increasing TCS levels with age and income	
Teitelbaum et al., 2008	Evaluate intra-individual temporal variability	New York City children (n=159) 6-10 years of age 2004 data collection	71.7% detection	Limited generalizability (only Black and Hispanic nationalities) Small sample size, limited power
Wolff et al., 2008	Prenatal exposures to phenol metabolites	US pregnant women (n=404) Single spot third-trimester sample 1998-2002 data collection	77.4% detection	One biomarker measurement in third trimester
Casas et al., 2011	Evaluate the extent of exposure to phenols (one spot urine sample in third trimester)	Spanish pregnant women (n=120) Single spot sample 2004-2008 data collection	59.5% detection	Small sample size, limited power
Clayton et al., 2011	Association of endocrine-disruption compounds on immune function	US general population 2003-2006 NHANES data ≥ 6 years of age	Increasing TCS levels with age and income	Cross-sectional design Small sample size, limited power
Kim et al., 2011	Exposure to triclosan	Korean adult population (n-1870) 18-69 years of age 2009 data collection	92.6% detection Increasing TCS levels with age	Single spot , lifestyle factors (levels lower than US population)
Li et al., 2011	TCS urinary levels	Chinese children and students (n=287) 3-24 years of age	93% detection Decreasing TCS levels with age	
Woodruff et al., 2011	Characterize individual and multiple chemical exposures	US pregnant women (n=86) 2003-2004 NHANES data ≥ 6 years of age Single spot sample	87% detection	Possibility of non-representative exposure patterns
Environmental Defence 2012	Canadian exposure to triclosan	General Canadian population (n=8)	Single spot sample, detected in 7/8 participants	Small sample size (n=8)
Philippat et al., 2012	Prenatal exposures to phenols	French pregnant women (n=191) Single spot third	84.1% detection	No record of time of sampling

		trimester sample 2002-2006 data collection		
Health Canada, 2013	Second report on human biomonitoring of environmental chemicals in Canada	Canadian population (n=2550) CHMS Cycle II, 2009-2011 3-79 years of age	71.80% detection	
Meeker et al., 2013	Determine distributions, variability, and predictors of urinary biomarkers of environmental phenols and multiple times during pregnancy	Northern Puerto Rico pregnant women (n=105) Spot urine sampling in each trimester 2010-2012 data collection	88.9% detection rate No difference between study visits Increasing TCS levels with age	Self-reported product use

3.4 RISK CHARACTERIZATION

Triclosan was detected in 76% of urine samples of Americans in the National Health and Nutrition Examination Survey (NHANES) conducted in 2003 in United States (Woodruff et al. 2011). Triclosan has been detected in 76% of liquid soaps and 29% of bar soaps in a national US survey conducted in 2001 by Perencevich et al. (Perencevich et al. 2001). Body burdens of TCS have been correlated with the use of products containing TCS as an active ingredient (Dodson et al. 2012; Fang et al. 2010).

Triclosan is an ingredient on Health Canada's Hotlist, a list which is used to communicate the names of prohibited and restricted cosmetic ingredients that may cause harm to the health of the user (Health Canada 2011c). In March of 2012, Health Canada and Environment Canada completed a preliminary assessment of TCS under the *Canadian Environmental Protection Act (CEPA), 1999* and the *Pest Controls Products Act*. It was concluded that "triclosan is not harmful to human health at current exposure levels, but in significant amounts can cause harm to the environment (Health Canada and Environment Canada 2012)". There was no clear evidence of a link between triclosan and antibacterial

resistance. As no Canadian data were available for this assessment, Health Canada used biomonitoring data from the US NHANES study to estimate the total Canadian exposure to TCS. During a 60 day comment period, Health Canada and Environment Canada proposed a voluntary reduction of the use of household products containing TCS (Health Canada and Environment Canada 2012). Subsequently, in May of 2012, Environmental Defence produced the first report of TCS concentration measurement in Canadian adults. They recommended "a mandatory ban on TCS in household products to protect the health of Canadians and the environment (Environmental Defence 2012)". They concluded that 87.5% of their volunteers had detectable urinary levels of TCS. Further information from the media report of this study, released on May 16th, 2012, is available in Appendix A. Since the release of the Environmental Defence report, certain companies have voluntarily decided to remove triclosan from their list of active ingredients. Most recently, *Johnson and Johnson* has removed it from all of their adult products (Kay 2013).

Currently, Health Canada has approved maximum TCS concentrations of 0.03% in mouthwashes and 0.3% in other cosmetics (Health Canada and Environment Canada 2012). These levels are consistent with those approved in the United States and Europe (Government of Canada 2013). Approved triclosan concentration levels are slightly higher (1.0%) in personal care products that are regulated as a drug (Government of Canada 2013). When these products are used as directed, triclosan is rapidly excreted from the body, creating a low chronic health risk and low human toxicity (NICNAS 2009; Rodricks et al. 2010).

In order to measure exposure to varying chemicals over time, biomonitoring techniques have been developed and are an important tool in risk assessment.

3.5 SUMMARY OF THE LITERATURE

The findings of this review show that triclosan is consistently detected in a high number of urine samples among children, adults and pregnant women in many countries around the world. There is some evidence that triclosan levels are positively associated with income (Calafat et al. 2008; Clayton et al. 2011), while there exists conflicting results regarding how triclosan levels change with age (Calafat et al. 2008; Clayton et al. 2011; Kim et al. 2011; Li et al. 2011; Meeker et al. 2013). The present study will attempt to contribute to the ongoing research in the field through identifying the main sources of exposure to triclosan, establishing predictors of urinary triclosan levels, measuring current urinary triclosan levels through biomonitoring, as well as through the development of a model to highlight the pattern of urinary triclosan concentrations following triclosan exposure.

CHAPTER 4: METHODS

4.1 STUDY DESIGN

Data for this thesis has been collected from The P4 Study: Plastics and Personal-Care Product Use in Pregnancy. The P4 Study is a Health Canada Chemicals Management Plan funded longitudinal observational study led by Dr. Tye Arbuckle, a Senior Epidemiologist and Research Scientist, working for the Healthy Environments and Consumer Safety Branch of Health Canada. The P4 Study examined exposure among a small group of Canadians to triclosan, phthalates, bisphenol A, naphthalene, cotinine and triclocarban at all stages of pregnancy and post-partum, while concurrently collecting questionnaire data and product use activity information. Advantages of longitudinal observational studies include allowing for investigation of events or changes that occur over time within the same individual. Also, each participant serves as their own control, thereby eliminating confounding due to between-subject variability.

This thesis covers the analysis of triclosan, one of the chemicals measured in the P4 Study.

4.1.1 Data/biospecimen collection

The P4 Study consisted of five study visits. Visit T1 occurred within the first 20 weeks of pregnancy. This visit consisted of two separate sub-visits, one on a week-day and the other on a week-end day. Visit T2 was a second trimester visit, while visit T3 was a third trimester visit. Visits T4 and T5 were post-partum visits, taking place right after the birth of the infant as well as approximately 2 – 3 months post-partum, respectively. At these repeated occasions throughout pregnancy, several different biomonitoring matrices were analyzed for presence of the numerous P4 study chemicals. These biomonitoring matrices included

maternal and infant urine, breast milk, meconium, and infant formula. Serial maternal urine samples were collected over a 24-hour period during the week-day and week-end day T1 Visit, while a single spot maternal urine sample was collected at Visit T2, T3, and T5. The biomonitoring data were recorded in biospecimen tracking logs, which can be reviewed in Appendix B.

In addition to the collection of biomonitoring samples, self-reported "Product Use Booklets" and "Food and Activity" diaries were also completed by participants for specific time periods coinciding with their urine sample collections provided at the various study visits. The women also completed questionnaires at each of the study visits during pregnancy and approximately 2 – 3 months post-partum.

The P4 Study staff performed a number of tasks throughout the duration of the study. In addition to collaborating with the clinical nurses, receptionists and ultrasound technicians, they were responsible for participant recruitment and informed consent, completion of participant case report forms, as well as the scheduling of home visits to pick up the urine specimens and the completed diaries.

A summary of the procedures and requirements of participants and staff for each of the study visits are detailed in Table 3.

Table 3. Summary of study visits. This table summarizes the study design, including all data collected at each time point. The blue boxes indicate participant tasks; the grey boxes indicate staff-facilitated tasks.

T1: Completed prior to 19 completed weeks gestation							
Study Period	Recruitment	**T1a (week-day)**			**T1b (week-end)**		
Place	Clinic	Home			Home		
Time Frame	Pre study	0 hrs	24-48hrs	At 48hrs	0 hr	24-48hrs	at 48hrs
Study Tasks	Consent Form T1 CRF Participant receives cooler bag, urine specimen cups, freezer packs, diaries	Begin diary	Continue diary 24 hrs of urine collection	Home Visit to pick up urine, and diary	Begin diary	Continue diary 24 hrs of urine collection	Home Visit to pick up urine, and diary

Study Period	**24-28 weeks T2**		**32-36 weeks T3**		**Delivery T4**	**2-3 mo. Postpartum T5**	
Place	Home	Clinic/Home	Home	Clinic/Home	Hospital	Home	Home
Time Frame	0 hrs	24 hrs	0 hrs	24 hrs	Delivery	0 hrs	24 hrs
Study Tasks	Begin diary	Spot urine sample CRF 2	Begin diary	Spot urine sample CRF 3	Chart Review (CRF 4)	Begin diary	Home visit to collect: Spot urine sample CRF 5

CRF: case report form questionnaire administered to study participants

4.2 SETTING AND STUDY POPULATION

4.2.1 Setting of the study

Ontario has a population of 13,505,900 (Ontario Ministry of Finance 2013). Canada's capital city, Ottawa, has a population of 883,391 (Statistics Canada 2012). The Ottawa Hospital combines the services of the General, Civic and Riverside Campuses. With over 12,000 staff members and more than 6,500 babies delivered from 2011-2012, it is one of the busiest hospitals in Ontario (The Ottawa Hospital 2012).

For the purpose of this study, the participants were all residents of Ottawa, while the physicians and research nurses worked at The Ottawa Hospital.

4.2.2 Study population

Eighty pregnant women were recruited for the study from the Ottawa Hospital between 2009 and 2010. Initial eligibility criteria included healthy women aged 18 years or older, in the first trimester of pregnancy, with a singleton, viable fetus, planning on delivering at the Ottawa Hospital, General Campus, and with the ability to consent and communicate in English or French. Women who were planning a home birth within the city were also deemed eligible for the study as an effort to boost enrolment.

4.2.2.1 Criteria for ineligibility

Women who had known fetal abnormalities (e.g. hydatidiform mole), known fetal chromosomal anomalies or major malformations in their current pregnancy were excluded from the study. Any women with a history of any of the following medical complications were also excluded: renal disease with altered renal function, thyroid disorder, hypertension, diabetes, epilepsy, any collagen disease such as lupus erythematosus and scleroderma, active and chronic liver disease (hepatitis), heart disease, serious pulmonary disease, cancer, haematologic disorder, threatened spontaneous abortion, and illicit drug use.

Exceptions to these exclusion criteria included women with anaemia or thrombophilia, or women who experienced bleeding in the first trimester provided that their chart documented a viable fetus at the time of recruitment (Tye Arbuckle, P4 Study Protocol, May 2009).

The most common reason for which potential research participants were not eligible for the study was that they were delivering at a hospital outside of Ottawa. As ultrasound clinics were not available in smaller centres, women from outlying areas needed to come into the city for their ultrasounds. It was at these clinics that the recruitment took place. Another common reason for ineligibility was many women had a gestational age greater than 20 weeks before the completion of the T1 visit. Other reasons for exclusion included living too far away from Ottawa for research staff to complete home visits, having serious co-morbidity, being too sick to take part in the study, and no response given. Figure 2 indicates the detailed reasons potential research participants were not eligible for study participation.

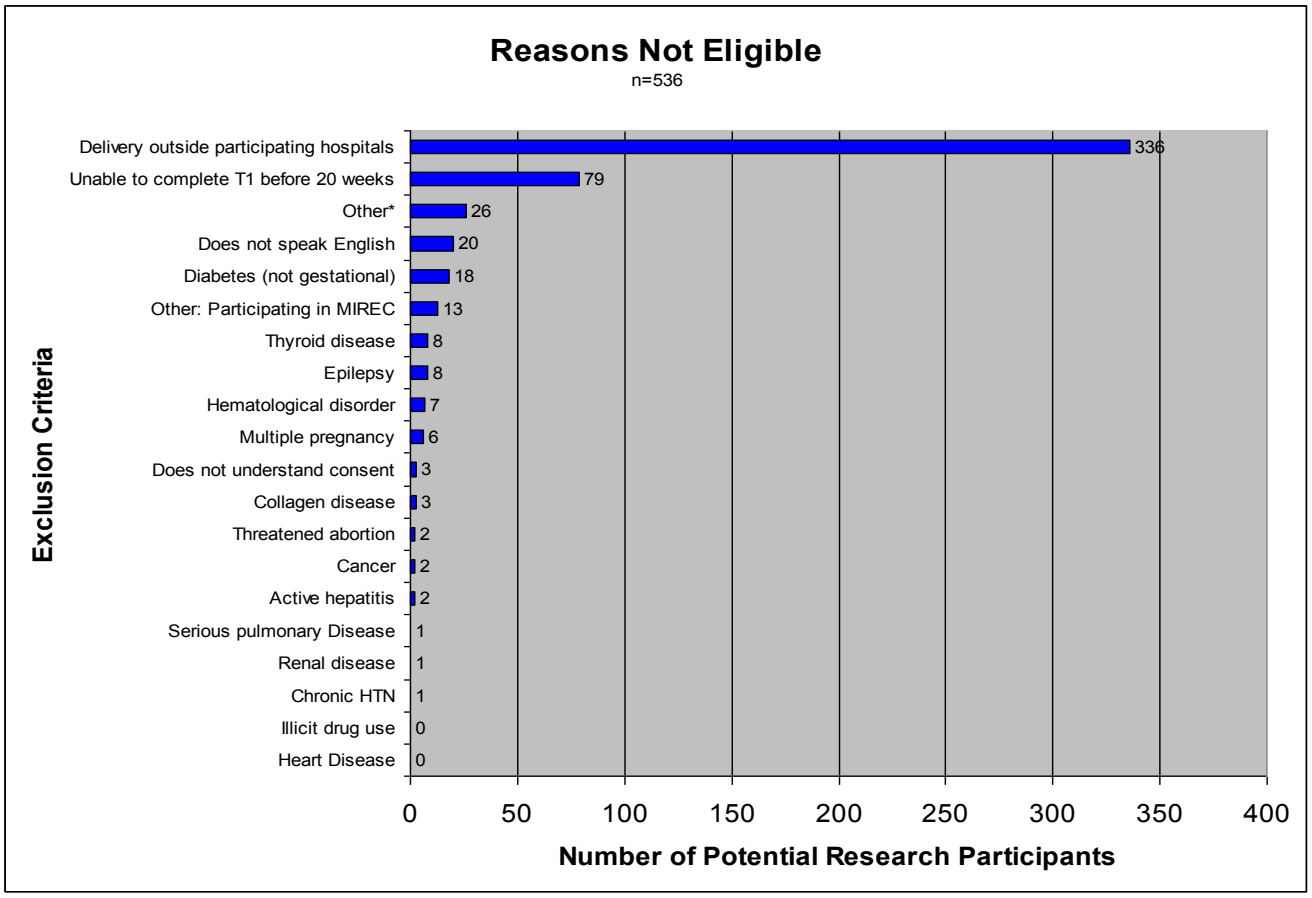


Figure 2. Reasons for study participant ineligibility.

4.2.3 Participant recruitment

Recruitment began in November 2009 in the obstetrical clinic at The Ottawa Hospital, General Campus, including only participants who planned to deliver at this hospital and were in the first trimester of pregnancy. Recruitment took place primarily through ultrasound clinics and high risk clinics; however, midwifery groups also played a role in study participant recruitment. The research study was presented to physicians at their weekly or monthly rounds, where the P4 Study staff formed collaborative relationships with physicians' support staff (nurses, receptionists, ultrasound technicians) to gain access to the patients. Physicians who spoke favourably to their patients about participation in research

studies highly assisted the recruitment. Research staff also met with family doctors at other offices within the community to inform them of the research study.

In February 2010, study posters and brochures were delivered to obstetrical and general practitioner's offices across the city. A copy of the study brochure is included in Appendix C. Special attention was made to design and poster attractiveness through complex and colourful graphics. Due to low recruitment, the study expanded the inclusion criteria in March 2010 to include women delivering anywhere in the city of Ottawa. This included hospital deliveries, as well as home births. At this time, recruitment began at a private obstetrical clinic called Harmony, developed through partnership with physicians at The Ottawa Hospital, Civic Campus. Recruitment at Harmony was from ultrasound clinics and regular obstetrician visits. In order to increase recruitment levels further, the top end of the gestational age limitation was increased in May of 2010 from 13 weeks 6 days to 19 weeks 6 days, and the compensation for participants' time was increased from $50 to $100. Many participants were ineligible because they could not complete the T1 visit before 20 weeks gestational age. It was common to encounter women who were between 18-22 weeks gestational age in the ultrasound clinics because this timing coincides with the morphology ultrasound. Although women who were 18 weeks gestational age were usually able to complete the T1 visit in time, it was common to explain the study to a potential research participant only to find that she was slightly past the gestational age range for inclusion. Figure 3 shows the cumulative participant recruitment by site and month throughout the study.

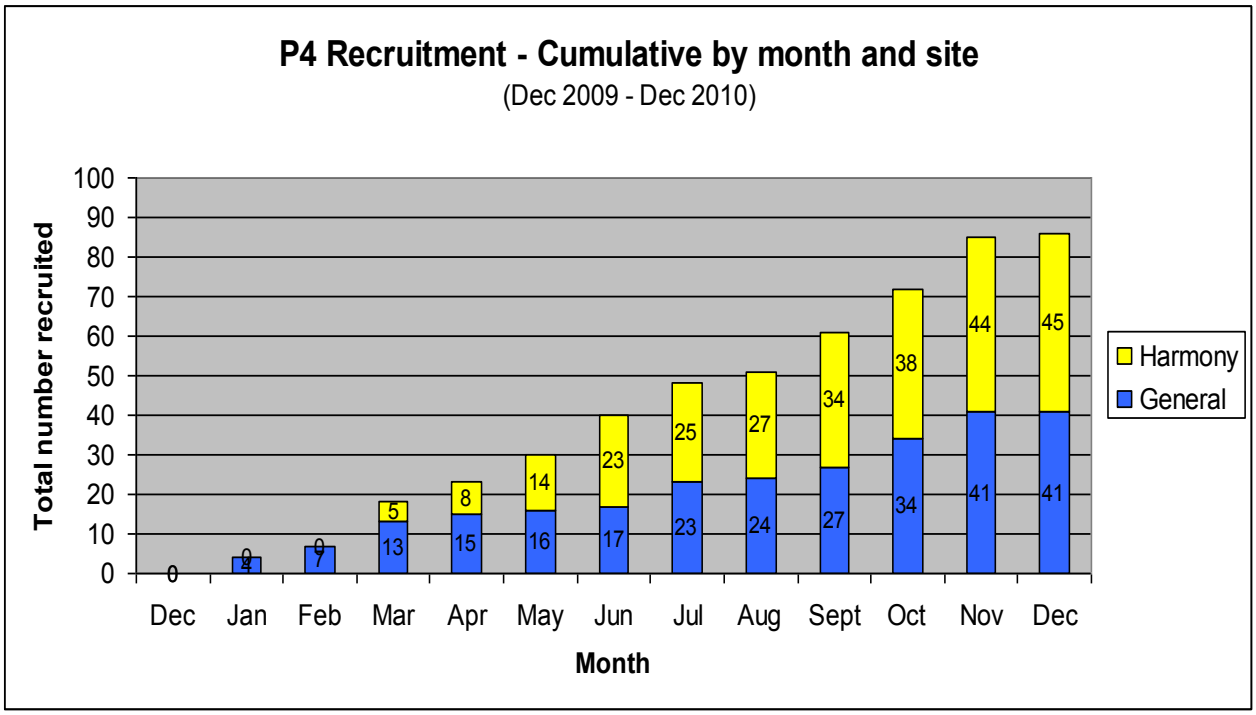


Figure 3. P4 cumulative participant recruitment by site and month from December 2009 to December 2010.

Overall, between November 2009 and December 2010, 86 healthy pregnant women from Ottawa, Ontario, Canada were recruited from physician and obstetrician offices, ultrasound clinics, and midwifery groups as participants of the P4 Study. Six women withdrew prior to the first study visit. As a result, our study sample consisted of 80 women.

Only 1307 women were assessed for study eligibility. As the number of births at the Ottawa Hospital during the study period was greater than 6500, this represents only approximately 20% of the sampling frame. Based on the criteria mentioned above, only 769 of the 1307 were eligible to take part in the P4 Study. The percentage of women who volunteered to participate was only 11% for this study (86 out of an initial 769 eligible participants) due to lack of interest. It is important to note that the questionnaire given to participants choosing not to volunteer for the study failed to give participants the options to

say “prefer not to answer” or “unsure”, so it is hypothesized that “not interested” was the default answer. Despite the low acceptance rate, the collaboration between the research and clinical staff resulted in an overall successful number of recruited study participants. The overall retention rate of study participants was 84% after excluding early outcomes such as neonatal death or miscarriage and medical withdrawals. Figure 4 shows the flow diagram of participant selection throughout the recruitment periods. “Lost to follow up” was defined as a missed visit without an active withdrawal. Hence, there was sometimes a higher participant number in subsequent visits. The numbers in the flow diagram do not necessarily add up because some participants missed one study visit but were present for the remaining visits. In order to assist with participant retention, participants in this study were contacted a minimum of every 2-3 months.

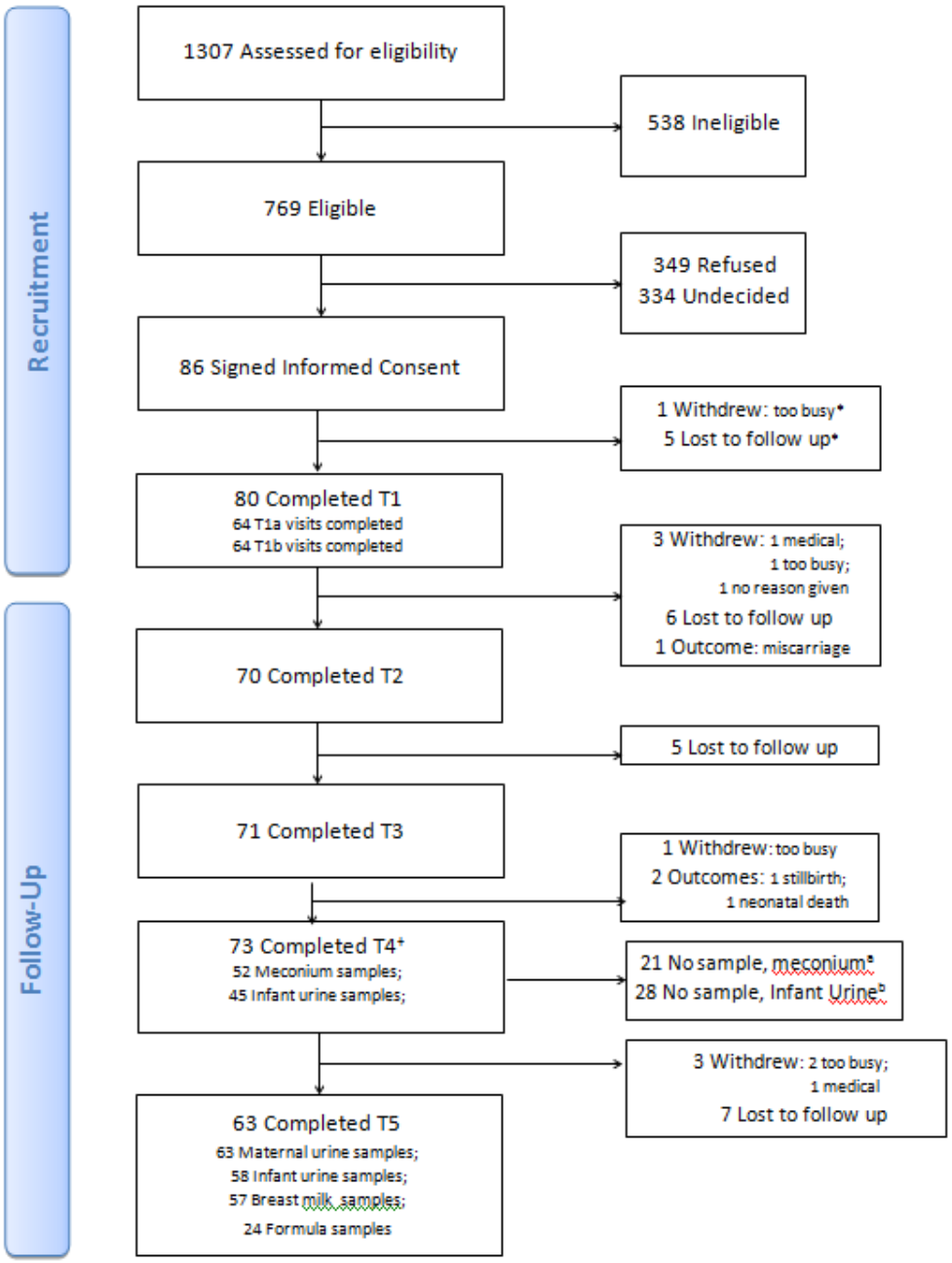


Figure 4. Participant flow diagram.

[+]Defines the number of participants remaining in the study after the neonatal period

[a] The term "No sample, meconium" includes all possible reasons meconium was not collected. This includes: Meconium present at birth therefore no meconium in diapers, patient did not save meconium, staff was not contacted at delivery, not enough meconium to scrape into tubes, stool and transitional stool

[b]The term "No sample, infant urine" includes all possible reasons infant urine was not collected. This includes: 2 or more unsuccessful urine bag attempts; the patient did not want this procedure done at this point; unable to contact the patient

Loss to follow up is defined as a missed visit without an active withdrawal.

4.2.4 Sample size and power calculation

When the P4 Study was initially designed, there was limited biomonitoring data available upon which to calculate sample sizes for many of the chemicals, including triclosan. As a result, study power calculations were performed using a common phthalate metabolite: mono-ethyl phthalate (mEP). There were a number of published biomonitoring studies on mEP which showed considerable variability in exposure. Phthalates tend to have a skewed distribution, so log-transformed mEP levels were modelled as normally distributed with mean 5.3 and variance 0.51, based on mean and standard deviation values of 259.8 and 212.8, respectively, as reported by Hoppin et al. (Hoppin et al. 2002). As reported in the P4 Study Protocol, the assumed mixed effects model is:

$$\log(mEP_{ij}) = \beta_i + \varepsilon_{ij}.$$

"mEP_{ij} represents the ith woman's measured level of mEP on the jth day, β_i represents the ith woman's average level of mEP and ε_{ij} represents random variation reflecting intra-subject variability. This model assumes that the different women's average levels were normally distributed as $\beta_i \sim N(\mu, T^2)$, where μ represents the overall average level of mEP and T^2 represents the inter-subject variance. Finally, the model also assumes that the individual variation term is normally distributed as $\varepsilon_{ij} \sim N(0, \sigma^2)$, where σ^2 represents the intra-subject variance. The overall variance of the log-transformed mEP levels would be $0.51 = T^2 + \sigma^2$, the sum of the inter-subject and intra-subject variance".

A simulation was performed with 1000 samples of 15 women, each with 6 urine spot tests throughout a day and with equal inter- and intra-subject variability ($\sigma = \tau$). Results of the power calculation concluded that a sample size of 80 women would provide 80% power

to estimate the variability within each individual with an error of less than 0.06, while accounting for any loss of data from study limitations mentioned above. Since the overall variance is 0.5, this means that our final estimate of between-subject variability will be reliable because it will not be biased by unaccounted-for between-subject variability (Tim Ramsay, P4 Study Protocol, May 2009).

4.3 DATA COLLECTION

4.3.1 Ethics, Informed Consent

The P4 study received ethical approval by the Health Canada Research Ethics Board and the Ottawa Hospital Research Ethics Board.

Individual written and signed consent was obtained for each participant. A copy of the consent form completed by each participant in the study is included in Appendix D. This information was collected by hospital research nurses, who undertook self-directed training to understand the study protocol and procedures, and to learn the procedures of patient screening, recruitment, informed consent, specimen and data collection and processing, as well as shipment of biospecimens. Study procedures were clearly explained and participant eligibility criteria were assessed by the research nurses. The participant eligibility form is included in Appendix E. All information and specimens provided by participants were coded with a unique participant identifier in order to preserve confidentiality of personal information.

Throughout pregnancy, all medical conditions and prescribed medications were recorded in each woman's medical chart. In addition, the outcome of the pregnancy was also recorded. This information was extracted from each participant's chart for the purpose of this study.

4.3.2 Maternal Urine Collection

Women were asked to provide multiple urine samples on five different occasions throughout their pregnancy and post-delivery. Serial urine samples were collected on two different occasions (week-day and week-end day) during early pregnancy (between 6 and 19 weeks). For these collections, participants were asked to collect each of their urine voids within a 24-hour time period to provide information on the temporal variability of urinary triclosan concentrations within a day. In addition, single spot samples (minimum of 50mL) were collected during the 2nd trimester (24-28 weeks), the 3rd trimester (32-36 weeks) of pregnancy, as well as two to three months postpartum. Urine samples were collected at the participant's home or at their regularly scheduled clinic visit with their obstetrician or family doctor. All post-partum samples were collected at the participant's home. In the event of home collections, a research assistant from The Ottawa Hospital personally collected each of the samples. In order to avoid degradation of the chemical, study staff were reminded that the urine must be kept cool (4°C) during the collection period, mixed well and aliquotted within 36 hours of collection, and then stored frozen at -80°C (Calafat et al. 2008). The maternal urine collection schedule is detailed in Appendix F.

Data from maternal urine sample logs, questionnaires and diaries were entered directly into MS Access 2007 using a standardized data collection form.

4.3.2.1 Biospecimen analysis

All laboratory analyses of the P4 biospecimens took place at the Centre de Toxicologie du Québec (CTQ), Institut national de santé publique du Québec (INSPQ). Triclosan was quantified in urine samples with the GC-MS-MS (gas chromatography coupled to tandem mass spectrometry) method, following a solvent extraction protocol. The detection limit was 3.0 μg/L. Field blanks of distilled water were aliquotted to test for

contamination during the process, and a 50mL field blank of deionised water for each participant was also analyzed. Specific gravity of the urine was measured by refractometry (Atago UG-α, Cat. # 3464) with automatic temperature compensation (ATAGO 2003-2013).

4.3.3 Questionnaires and Exposure Journal

Upon recruitment, participants completed a questionnaire collecting details on demographics, socio-economic status, employment, smoking history, obstetrical history, current pregnancy information, and information on potential sources of exposure to the chemicals of interest. Participants were also asked to complete additional questionnaires throughout pregnancy as well as postpartum. These questionnaires collected updated pregnancy, employment, smoking history, and exposure information. A chart review questionnaire was also completed for each participant containing information on health problems during pregnancy, and any medications prescribed to the woman during her pregnancy.

Both a "Food and Activity" diary and a "Product Use Booklet" were given to each participant to complete at specified times throughout their pregnancy. These can be found in Appendix G. In particular, for the first two serial urine sample collections, participants completed the journals over a 48-hour period starting 24 hours prior to commencing the serial urine collection and continuing throughout the 24-hour collection. For the single spot urine samples collected in the second and third trimester, as well as approximately 2 – 3 months post-partum, women only completed the journals for the 24-hour period prior to the collection of the spot urine sample. The complete data collection schedule is detailed in Appendix F.

In the "Food and Activity" diary, all foods and drinks consumed, and personal-care products used throughout the day were recorded, including the time of use for the pre-

specified time period. Also included in these journals were notes of any missed urine collections and any medical procedures throughout pregnancy that were close in time to the collection of the urine samples, such as amniocentesis, dental work, or blood draws. Meanwhile, in the "Product Use Booklet", women recorded detailed product and brand name information including dates consumed/applied for all products listed in the "Food and Activity" journal. Questionnaire data, as well as food, activity, and product information data were entered directly into a MS Access 2007 database.

Throughout the course of the study, many of the Product Use Booklets (PUBs) were not returned. Out of 80 participants, 53 PUBs were returned, 19 were lost and there is no available information on the remaining PUBs from participants with early outcomes such as miscarriages or neonatal deaths, as well as from those who were lost to follow-up. As a result, the brand name and manufacturer of the products that they used at any point in the study are unknown for some of the women.

4.4 DEFINITIONS OF VARIABLES

The **dependent variable** or primary outcome is urine triclosan concentrations (μg/L) in pregnant women, as measured by both single spot urine and serial 24-hour urine samples. All urine samples with a volume of 50mL or greater were included in the analysis. Un-recorded voids were coded as missing.

The **independent variable** of personal care product use summary score is a continuous variable representing the extent of exposure of an individual to products containing triclosan. Each person received an individual summary score based on the sum of the total number of exposures to an individual personal care product throughout their pregnancy.

Product categories for the classification of triclosan include:

Category I: **Cosmetics**: products used for making up the eyes, face, or lips

Category II: **Hair care**: products used for cleaning, treating, conditioning and styling of hair

Category III: **Oral care**: products used for dental hygiene

Category IV: **Baby products**: products that are applied to a baby

Category V: **Deodorant/Antiperspirant**: products used for underarm hygiene

Category VI: **Hand soaps/sanitizers**: products used for washing hands

Category VII: **Lotions/Creams**: products used for moisturizing hands, face, body, and feet

Category VIII: **Skin care**: products that are used as cleansers and toners for the face, body and feet

Category IX: **Medication/vitamins**: products that are used for medical treatment or health maintenance

Category X: **Household cleaning products**: products that are used for household disinfection

Category XI: **Other products**

Additional **covariates** included in the study models were categorized as follows, ensuring a minimum sample size of 5 participants per category for each categorization.

- Marital age:

(1=<30 years / 2=30-34 years / 3=35-39 years / 4=≥40 years / 99= “missing”)

- Education:

(1=High School diploma, some College or University classes / 2=College diploma / 3=Undergraduate University degree / 4=Masters or PhD)

- Marital status:

(1=Married / 2=Single, divorced, separated, widowed, or living with partner for greater than one year)

- Combined household income:

(1=<60,000 / 2=60,001-80,000 / 3=80,001-100,000 / 4=>100,000 / 99= "missing, refuse, don't know")

- Country of birth:

(1=Canadian / 2=Non-Canadian)

- Time of day of urine void collection:

(1=00:00-07:59 / 2=08:00-15:59 / 3=16:00-23:59)

- Parity: the number of times a woman has been pregnant for 20 or more weeks regardless of whether the infant is dead or alive at birth (current pregnancy excluded) (Centers for Disease Control and Prevention, 2011)

(0=none/1=once/2=two or more times)

- Season of sample: season in which urine sample was collected

(1=spring / 2=summer / 3=autumn / 4=winter)

- Season of conception: season in which conception took place, as determined through gestational age information provided in study visit questionnaires

(1=spring / 2=summer / 3=autumn / 4=winter)

Season was defined according to the specific dates corresponding to each season for the years of the study sampling:

- Spring: March 20 – June 20, 2010; March 20 – June 20, 2011
- Summer: June 21– Sept 22, 2010; June 21 – Sept 22, 2011
- Autumn: Sept 23 – Dec 20, 2010; Sept 23 – Dec 21, 2011
- Winter: Dec 21 – Mar 19, 2010; Dec 21 – Mar 19, 2011

Also included as covariates were the total volume of the urine sample (in mL), as well as time since the last urine void (in seconds). Although study participants recorded their time since last urine void in hours and minutes, seconds was selected as the units for the time since last void variable in the study model due to ease of coding within the Statistical Analysis Software (SAS) 9.3 program.

4.5 DATA CLEANING

This thesis consisted of analyses of secondary datasets, collected from the P4 Study. These final datasets were available as of May 2012. One of these datasets included all the laboratory and biospecimen tracking log information, which contained 55 different variables, some of which being the participant identifier numbers, barcode numbers for each metabolite sample, sample collection dates and times, laboratory measurements of each of the study metabolites, and adjustment factor measurements of specific gravity and creatinine. Cleaning of this dataset was performed for the purpose of this thesis in order to obtain a final dataset containing only the laboratory information relevant to triclosan.

A second dataset was also available containing all of the demographic information collected from participant questionnaire data. Each of the variables was labelled as either categorical or continuous. The categorical demographic variables were grouped into categories, ensuring a minimum of 5 entries per cell; the dataset was coded accordingly.

The laboratory dataset and the demographic information dataset were then merged into one final dataset, which was then prepared for analysis using Statistical Analysis Software (SAS) 9.3 (SAS Institute, Cary, NC).

Lastly, a partially complete dataset was available, containing questionnaire information, as well as information from the "Food and Activity" diaries and the "Product Use Booklets". Further data entry was required to complete the dataset. In the end, there were many cases in which the information provided in the diaries did not match that of the booklets. For example, an activity of "brushing teeth" may have been recorded in the diary; however, there was no corresponding recording of "toothpaste" in the product booklet. Consequently, there was a high amount of missing data in this product dataset. After consulting incomplete diaries on a case-by-case basis and finding instances where use of a specific product by an individual had been identified earlier in the diary but not as specifically later (e.g., "Colgate Total toothpaste" earlier and then "toothpaste" later), imputation was performed. An additional 170 products and corresponding activities were added to the dataset to produce a final total of 6031 product and activity recordings. The final product dataset contained participant identifier numbers, activity information, product brand and manufacturer information, as well as the corresponding dates and times of each of the product uses. Each entry in this dataset was then individually categorized into one of the 11 product categories. Further classification placed each of the entries into a category of "product contains triclosan", "unsure as to whether product contains triclosan", or "product does not contain triclosan". This dataset was then prepared for SAS analysis.

4.6 DATA ANALYSIS

Data were imported from MS Access 2007 into Statistical Analysis Software (SAS) 9.3 (SAS Institute, Cary, NC). The complete components of the database are listed in Appendix H. Components of interest for this thesis include questionnaires, journals, product use booklets, biospecimen tracking logs, and maternal urine specimen results from the laboratory at the Institut National de Santé Publique du Québec (INSPQ).

Previous studies analyzing triclosan concentrations in urine found that the distribution of TCS concentration was not normal. As a result, the variable required transformation (Calafat et al. 2008; Casas et al. 2011; Kim et al. 2011; Teitelbaum et al. 2008; Wolff et al. 2008). An initial analysis for normality in this data suggested that the urinary triclosan levels were positively skewed. As a result, this variable was log-transformed, and was included as such in the models which will be presented in the subsequent sections.

4.6.1 Adjustment of Maternal Urine Samples for Dilution Effect

Consideration of the hydration status of the individual is important for interpreting chemical concentrations in urine. Specific gravity adjustment has been labelled as a useful alternative to creatinine adjustment (Berlin et al. 1985; Haddow et al. 1994; Miller et al. 2004), especially in pregnancy (Adibi et al. 2008). Hence, urinary concentrations were adjusted for specific gravity, using the following formula, adapted from Just and colleagues (Just et al. 2010):

$$Pc = Pi\ [(SGm - 1)/(SGi - 1)],$$

where Pc is the SG-adjusted metabolite concentration (ng/ml), Pi is the observed metabolite concentration, SGi is the specific gravity of the urine sample, and SGm is the median SG for

the cohort. Both unadjusted and specific gravity adjusted concentrations will be reported for all calculations.

4.6.2 Descriptive Statistics

Descriptive statistics were used to describe the study population, the number of urine samples provided by each participant, and the distribution of participants in the various categories of maternal age, education, marital status, combined household income, country of birth, time of day of urine void, season of sampling, season of conception, and parity variables. In addition, the overall geometric mean and 95% confidence intervals, median, range and selected percentiles of urinary triclosan levels per participant in these categories were also calculated. No participants were eliminated from subsequent analyses based on their failure to provide urine samples.

The total percentage of samples in which the urinary triclosan level was greater than the limit of detection (LOD), corresponding to 3.0µg/L, as reported in the 2012 version of the INSPQ P4 laboratory summary report (LeBlanc and Marchand, 2012), was recorded. It was not necessary to employ a method to account for urinary triclosan concentrations that were below the LOD due to the ability of the lab results to quantify levels in samples below 3.0µg/L. For the purpose of study analysis, a constant of 0.0001 was added to each of the urinary triclosan concentrations of 0µg/L.

4.6.3 Product Use

With the abundance of products on the market, presence of TCS in the recorded products was identified with the assistance of the *White Paper* prepared by The Alliance for the Prudent Use of Antibiotics and the Health Canada online Drug Product Database (Alliance for the Prudent Use of Antibiotics 2011; Health Canada 2011a).

The eleven product categories were derived upon consultation of previous product classification methods for chemicals of emerging concern (Dhanirama et al. 2012; Koniecki et al. 2011; Romero-Franco et al. 2011). Categories included cosmetics, hair care, oral care, baby products, deodorant/antiperspirant, hand soaps/sanitizers, lotions/creams, skin care, medication/vitamins, household cleaning products, and other products.

Analysis of self-completed journals, product-use booklets and of questionnaire data in combination with the product categorization allowed for identification of which product categories were most frequently used by the participants, how product use varied across pregnancy, and the association between triclosan product use and triclosan geometric mean urine concentration levels.

4.6.4 Prediction of Urinary Triclosan Levels

A question of interest to this study was to determine whether any covariates significantly predicted urinary triclosan levels. Due to the correlations among the repeated urine sample measurements per participant in this study design, linear mixed effects models were fitted using the MIXED procedure in SAS, as has previously been done in similar studies (Hauser et al. 2004; Mahalingaiah et al. 2008; Teitelbaum et al. 2008). These models allowed the first urinary triclosan level of each participant to vary, while also allowing for variation in their responses over time. The spatial power covariance structure inherent in these models accounted for a change in correlation of responses over time; it was selected for its ability to handle the unbalanced observations, specifically the fact that the time points were unequally spaced, and were different across study participants.

Three different models were fitted:

1. The outcome was unadjusted log-transformed urinary triclosan concentration; specific gravity was not adjusted for nor included in the model in any way.

2. The outcome was unadjusted log-transformed urinary triclosan concentration; specific gravity was included as a covariate in the model.
3. The outcome was specific gravity adjusted log-transformed urinary triclosan concentration.

As previously mentioned, a constant value of 0.0001 was added to any urinary triclosan concentrations of 0µg/L within the dataset, in order to retain these samples in the analysis when log transforming the urinary triclosan concentrations.

Covariates included in each of the fitted models were: study visit, total volume, marital status, education, combined household income, country of birth, time of day of urine void, age, season of conception, season of sampling, parity, and time since last urine void. Type 3 tests of fixed effects of covariates will report associations between selected covariates and the log-transformed urinary triclosan levels. The numerical value of the Akaike Information Criterion (AICC), a measure of model quality, will be used to determine the most appropriate model for our study data.

Within-subject variance provided an estimate of the measurement error involved in using a single spot sample to estimate a subject's average exposure. Between-subject variance provided an estimate of the variability among urine samples provided by each of the different study participants.

The temporal variability of TCS within a 24-hour period following exposure and throughout pregnancy were assessed using the intra-class correlation coefficient (ICC), a measure of reproducibility, calculated by dividing the between-subject variability by the sum of the between- and within- subject variability. The value of the ICC summarized how well groups of observations at each time point resembled each other.

Any factors that predicted urinary triclosan levels were also identified. Tukey's HSD (Honestly Significant Difference) test was performed in combination with an estimate model statement to test for differences in mean concentration values between group categories. The Tukey's HSD test was performed in order to obtain an output which adjusted for the multiple comparisons, which could have increased the chance of Type I error.

Because the urinary triclosan levels were non-normally distributed (as was determined by Quantile-Quantile plots), non-parametric tests were performed on the log-transformed urinary triclosan concentrations. A Mann-Whitney test was performed to test for differences in week-day and week-end day urinary triclosan levels, while a Kruskal-Wallis test was used to test for differences in triclosan levels across pregnancy.

4.6.5 Predictive Ability of a Single Spot Urine Sample

In order to examine the predictive ability of the single spot samples taken throughout pregnancy versus a 24 hour urine collection sample, a surrogate categorical analysis was performed, as was done in similar studies (Braun et al. 2012; Hauser et al. 2004; Teitelbaum et al. 2008).

Terciles (low, medium, and high levels) of urinary TCS concentration levels were created based on cut-points determined by the distribution of each participant's geometric mean concentration levels.

The geometric mean value of specific gravity (SG) adjusted triclosan concentrations across all samples provided across study visits was calculated for each participant. This value classified them into one of the terciles, corresponding to the "observed true value" for that participant. In the case of serial urine samples provided in visits T1A and T1B, all individual urine samples were used in the calculation of the overall geometric mean for each participant.

The values of each individual SG-adjusted urine sample classified participants into one of the pre-determined terciles; each classification was referred to as the "predicted value".

Contingency tables were created to determine the sensitivity of single spot classifications to correctly predict an individual's overall exposure throughout pregnancy. Specifically, the overall agreement between the predicted and observed true classifications for each sample, as well as the agreement between study visits and between different times of day of urine sample collections were calculated. Chi-square tests of independence were performed to test whether a significant difference existed between the levels of overall predicted and true classifications.

4.6.6 Pattern of Urinary Triclosan Concentration Following Triclosan Product Exposure

In order to assess the pattern of urinary triclosan concentration following exposure to a product containing triclosan, a parametric linear trend model was fit. Specific gravity adjusted log-transformed urinary triclosan concentration was the outcome (dependent variable) and the time in hours since exposure to a product containing triclosan was the predictor (independent variable). The time since exposure variable was created using each individual's recorded time of exposure to a product containing triclosan, and their subsequent recorded urine samples provided in the 48 hours following the exposure.

CHAPTER 5: RESULTS

5.1 SAMPLE CHARACTERISTICS

The P4 study sample included 80 healthy, pregnant women. Amongst these women, the mean maternal age was 32.8 years, with a standard deviation of 4.70. Ages ranged from 19 to 47 years.

Table 4 reports sample characteristics by demographic category. Among the 80 participants, 36 (45%) were between the ages of 30 and 34 years, 36 (45%) had an Undergraduate University Degree, 63 (78.8%) were married, 44 (55%) had a combined household income of greater than $100,000, while 63 (78.8%) were born in Canada. Forty-six percent of study participants had no previous pregnancies prior to the pregnancy of interest for the study. Season of conception was equally distributed among seasons; the lowest percentage of women (14%) conceived in autumn.

Table 4. Demographic covariates of P4 Study participants (n=80) for all categories of maternal age, education, marital status, combined household income, country of birth, parity, and season of conception. Frequency and percentages of participant distribution.

	Frequency	Percentage
Maternal Age		
<30	17	21
30-34	36	45
35-39	19	24
≥40	7	9
Missing	1	1
Education		
< College Diploma	9	11
College Diploma	14	18

Undergraduate University Degree	36	45
Masters or PhD	21	26
Marital Status		
Married	63	79
Other	17	21
Income		
<60,001	7	9
60,001-80,000	11	14
80,001-100,000	13	16
>100,000	44	55
Missing	5	6
Country of Birth		
Canada	63	79
Other	17	21
Parity		
0	37	46
1	34	43
≥2	9	11
Season of Conception		
Spring	20	25
Summer	23	29
Autumn	11	14
Winter	26	32

The mean number of urine samples provided by an individual participant was 15.59 samples. The number of total urine samples provided by an individual ranged from 4 to 23.

5.2 EVIDENCE SUPPORTING EACH OF THE RESEARCH OBJECTIVES

Biospecimen tracking log data were merged with laboratory data. Product and activity information formed a separate dataset. Each was analyzed with Statistical Analysis Software (SAS) 9.3 (SAS Institute, Cary, NC).

5.2.1 Objective 1: To highlight the main personal care product sources of exposure to triclosan and their association with urinary triclosan concentrations.

Overall, there were 6031 product uses recorded by the study participants. Amongst the 11 product categories, the mean number of product uses per category by a participant was 9.0 with a standard deviation of 12.9. Participants used as little as zero products from a product category to as high as 60 products within one product category throughout pregnancy. The product categories most frequently used were "hand soaps/sanitizers" (905 product uses, 15%), as well as "lotions/creams (875 product uses, 14.5%). The fewest number of products were those of "baby products" (202 product uses, 3.4%), as well as "other" (176 product uses, 2.9%). Examples of products that were in the "other" category included bandaids and shaving gel. Figure 5 shows the distribution of all the product uses by product category. The number of products used per participant did not vary across pregnancy ($F=1.42$, $p=0.27$).

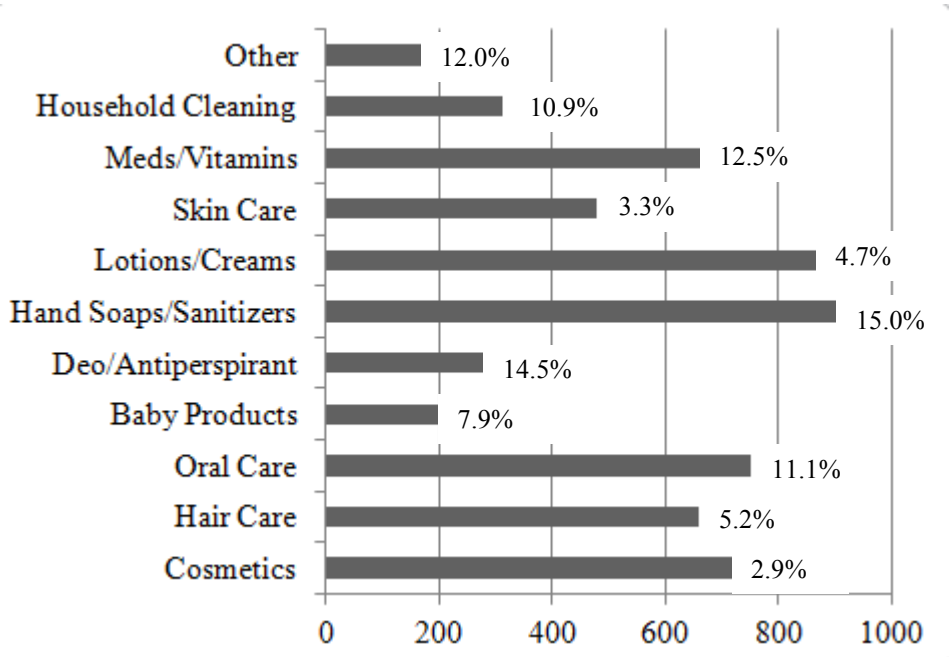


Figure 5. Total of personal care product uses by product category.

Of the 6031 total products used by the study participants, 266 products (4.41%) contained triclosan. Approximately a quarter of the products did not contain triclosan (4274 products, 70.87%), while for 1491 products (24.72%), it was not possible to determine whether or not the product contained triclosan due to insufficient information provided in the "Product Use Booklet". Figure 6 demonstrates the triclosan classification of the total recorded products by study participants.

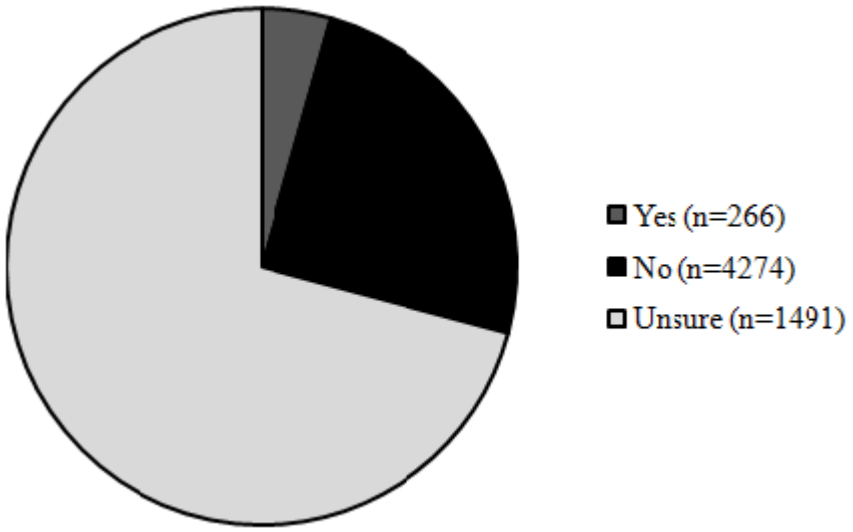


Figure 6. Triclosan classification of all recorded products used by study participants.

The number of triclosan product uses per participant ranged from 1 to 40 uses. The average was 7.51 with a standard deviation of 7.95, while the median number was 5 triclosan product uses.

Triclosan products were classified into product categories. 123 (46.6%) were "oral care" triclosan products, while 112 (42.4%) were "hand soaps/sanitizers" containing triclosan, totaling 89% of all the recorded products. Other categories containing triclosan products were "skin care" (5.3%), "household" (1.1%), and "other" (3.4%). Figure 7 shows the breakdown of triclosan product categorization.

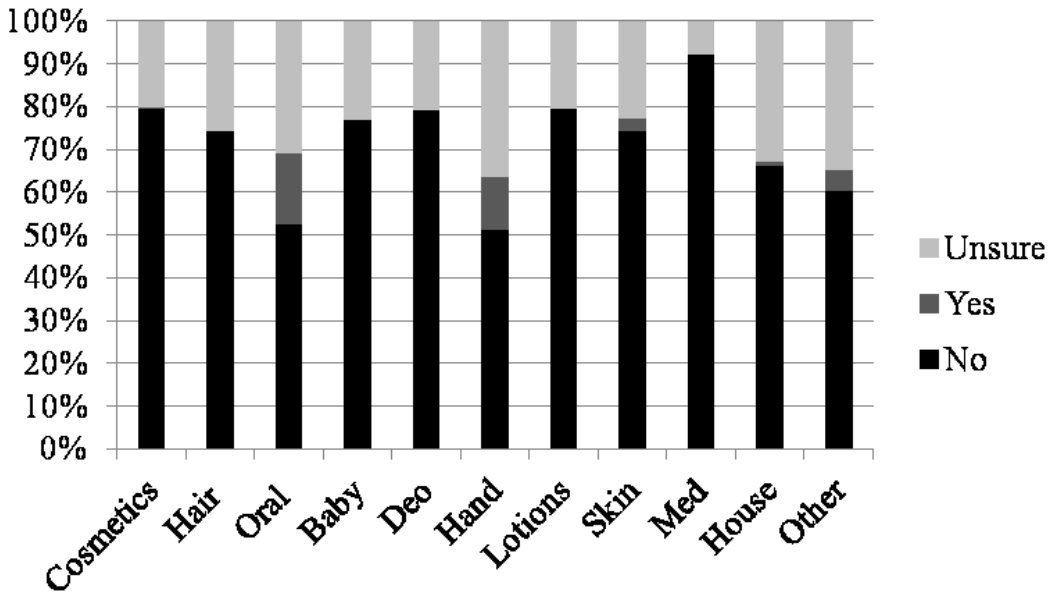


Figure 7. Triclosan products by product category.

There was a statistically significant positive correlation between the total number of triclosan product uses per participant and their individual specific-gravity adjusted geometric mean urinary triclosan level ($r=0.53$, $p=0.0009$).

5.2.2 Objective 2: To identify covariates which are statistically significant predictors of urinary triclosan levels.

Three linear mixed effects models were performed to test for associations between various covariates on urinary triclosan levels. Model 1 tested associations of various covariates on unadjusted urinary triclosan levels with no inclusion of specific-gravity in the model. Model 2 is identical to Model 1; however, specific-gravity is included as a covariate in the model. Model 3 tested associations of various covariates on specific-gravity adjusted urinary triclosan levels. Results of tests for fixed effects are listed in Table 5.

Table 5. Type 3 tests of fixed effects of covariates and Akaike Information Criterion (AICC). Excluding specific-gravity (Model 1), including specific-gravity as a covariate (Model 2) and adjusting the dependent variable for specific-gravity (Model 3). Linear mixed effects models. *P-value significant at <0.05.

	Model 1	Model 2	Model 3
Specific-gravity	n/a	F=111.53; p<0.0001*	n/a
Visit	F=0.84; p=0.499	F=1.30; p=0.267	F=1.11; p=0.351
Total volume	F=4.55; p=0.033*	F=0.34; p=0.561	F=0.24; p=0.624
Marital status	F=2.96; p=0.090	F=3.63; p=0.062	F=3.82; p=0.055
Education	F=1.75; p=0.166	F=2.32; p=0.085	F=2.04; p=0.118
Income	F=1.90; p=0.121	F=2.12; p=0.089	F=2.00; p=0.106
Country of birth	F=0.07; p=0.794	F=0.21; p=0.646	F=0.21; p=0.648
Time of day	F=5.66; p=0.004*	F=11.20; p<0.0001*	F=7.46; p=0.0006*
Maternal age	F=0.52; p=0.722	F=0.31; p=0.870	F=0.34; p=0.849
Season of conception	F=1.09; p=0.359	F=1.30; p=0.282	F=1.38; p=0.257
Season of sampling	F=3.08; p=0.027*	F=3.91; p=0.009*	F=3.35; p=0.019*
Parity	F=3.05; p=0.055	F=3.42; p=0.039*	F=3.45; p=0.038*
Time since last void	F=9.59; p=0.002*	F=0.72; p=0.396	F=1.13; p=0.287
AICC Information Criteria	4725.1	4612.4	4693.8

Time of day of urine collection and season of urine sampling were consistently significant predictors of urinary triclosan levels (Time of day: Model 1: p=0.004; Model 2: p<0.0001; Model 3: p=0.0006; Season of sampling : Model 1: p=0.027; Model 2: p=0.009;

Model 3: p=0.019). Each of the three models highlighted slightly different overall predictors:

Model 1 also identified total volume of the urine sample (p=0.033), and time since last urine void (p=0.002) as significant predictors of urinary triclosan levels. Model 2 identified specific gravity as a significant covariate ($p<0.0001$). Both Model 2 and Model 3 identified parity (Model 2: p=0.039; Model 3=0.038) as a significant predictor of urinary triclosan levels.

All three models reported similar measures of information criteria, indicating that each of the three models is of similar quality. Estimates of specific-gravity adjusted urinary triclosan levels by time of day category, season of urine sampling and parity, as incorporated in Model 3 are represented in Figures 8, 9, and 10, respectively. Geometric mean triclosan levels were significantly lower in urine samples collected between 16:00-23:59 than in samples collected from 09:00-15:59 or from 00:00-08:59. Urinary triclosan levels collected in autumn were 1.44 times greater than samples collected in winter. Women with no previous pregnancies had urinary triclosan concentrations 6.04 times greater than women with 2 or more previous pregnancies.

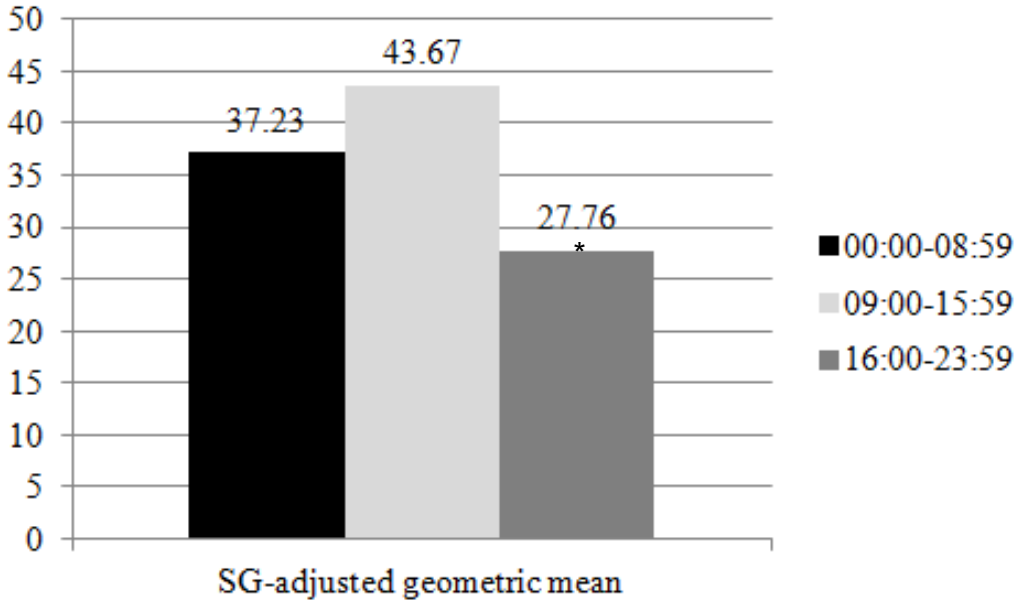


Figure 8. Geometric mean of specific gravity adjusted maternal urinary triclosan levels (µg/L) by time of day of urine sampling. * $p < 0.05$.

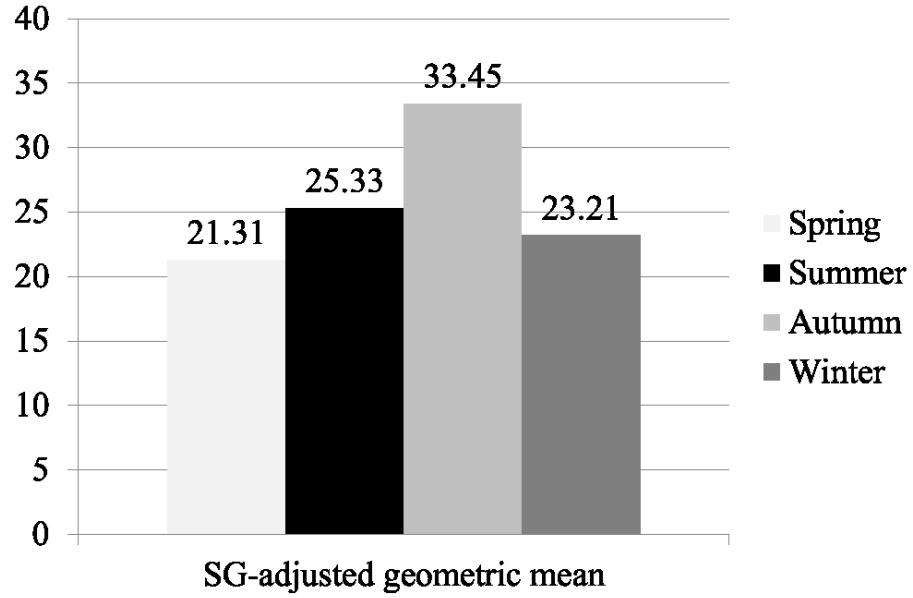


Figure 9. Geometric mean of specific gravity adjusted maternal urinary triclosan levels (µg/L) by season of urine sampling.

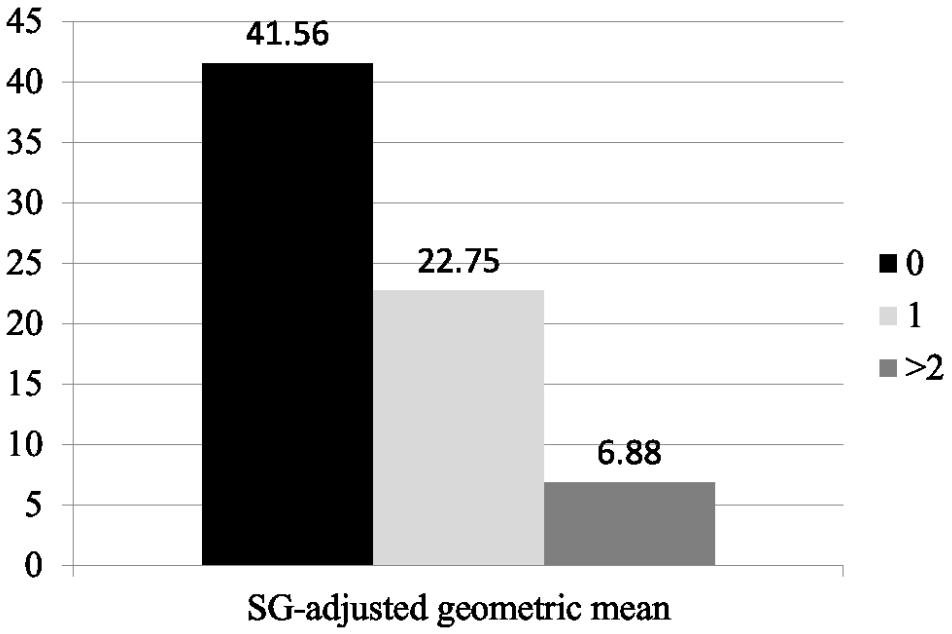


Figure 10. Geometric mean of specific gravity adjusted maternal urinary triclosan levels (µg/L) by parity.

5.2.3 Objective 3: To measure current exposure levels through urine biomonitoring.

5.2.3.1 Objective 3a: To measure inter-subject and within-subject variability of urinary triclosan levels.

The mean number of urine samples provided by an individual participant across the duration of the study was 15.61 (standard deviation: 5.20).The number of total urine samples provided by a single individual throughout the duration of the study ranged from 4 to 23. Overall, there were a total of 1247 maternal urine samples provided. The percentage of these samples which contained specific gravity adjusted urinary triclosan levels greater than the limit of detection (3.0µg/L) was 86.8%. Among all 80 study participants, 39 (51.25%) had at least one urine sample with a detectable level of triclosan (>3.0µg/L). As no triclosan was detected in any of the field blanks, potential contamination from the study collection materials, processing or storage conditions was not a concern.

Among maternal urine samples, the specific-gravity adjusted geometric mean triclosan concentration and the respective 95% confidence interval (CI) was 34.1µg/L (30.5-38.0), while the unadjusted geometric mean and 95% CI was 32.1µg/L (28.7-36.0). The maximum specific-gravity adjusted triclosan measurement was 2452.4µg/L, while the maximum unadjusted triclosan measurement was 3229.3µg/L. In addition to these values, the minimum and maximum concentrations, as well as the 10^{th}, 50^{th}, 90^{th}, and 95^{th} percentiles of TCS levels in all the maternal urine samples are described in Table 6.

Table 6. Geometric mean and selected percentiles of triclosan concentrations in maternal urine [µg/L (95% CI)].

	Specific-Gravity Adjusted	**Unadjusted**
No. of Samples	1247	1247
Geometric Mean	34.1 (30.5-38.0)	32.1 (28.7-36.0)
Minimum Value	0	0
10^{th} Percentile	2.4 (2.1-2.7)	2.2 (1.9-2.4)
50^{th} Percentile	23.3 (20.1-26.8)	25.3 (21.2-29.6)
90^{th} Percentile	526.4 (466.2-576.2)	523.2 (471.3-591.7)
95^{th} Percentile	774.9 (673.6-880.8)	833.4 (740.7-918.1)
Maximum Value	2452.4	3229.3

CI, confidence interval.

LOD for urine: 3.00µg/L

Results based on raw data provided by chemist (ignoring LODs)

The intraclass correlation coefficient for all urine samples was 0.24, indicating poor reproducibility among samples (Rosner 2006).

The overall distribution of the individual participant geometric means, illustrating the within-participant variability, is represented in Figure 11.

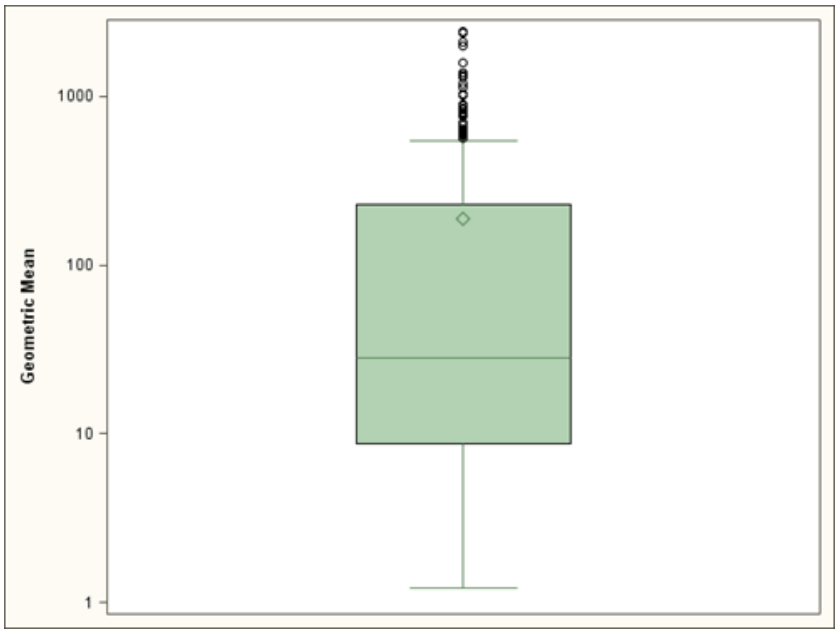


Figure 11. Boxplot of the specific gravity adjusted geometric means (µg/L) of all individual maternal urine samples. Y-axis log log base 10 scale.

There was no statistically significant difference between the overall geometric mean maternal urinary triclosan levels on a week-day (T1A) versus a week-end day (T1B) (χ^2=0.0026, p=0.96). Results are reported in Figure 12.

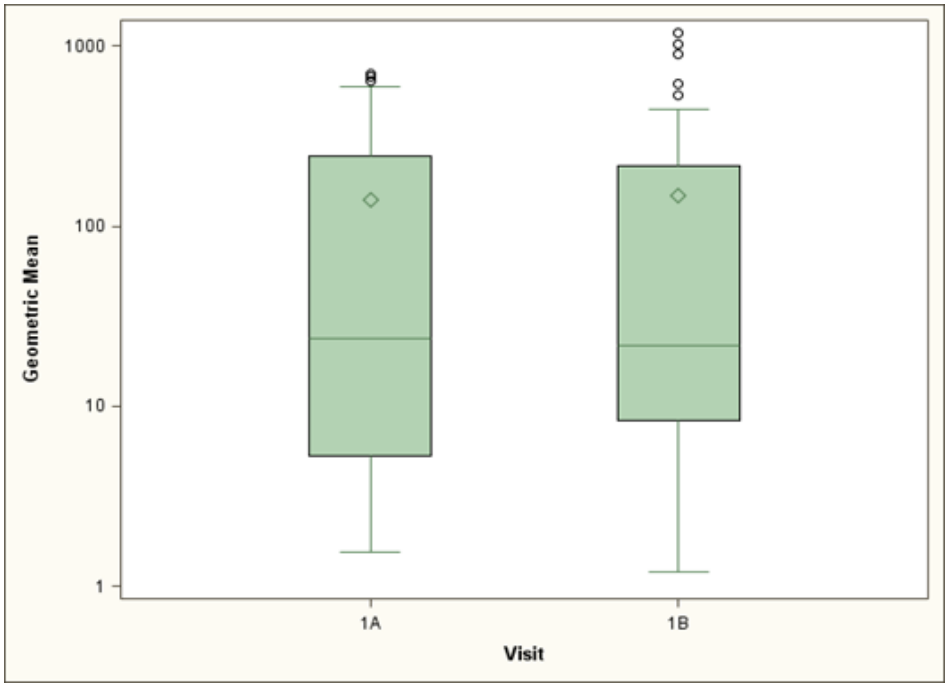


Figure 12. Boxplot of the specific gravity adjusted geometric means (µg/L) of all individual maternal urine samples by week-day collection (1A) and week-end day collection (1B). Y-axis log log base 10 scale.

Because no statistically significant difference was found between the samples collected on a week-day and a week-end day, these two study visits were combined into one visit (T1). There was no statistically significant difference between the geometric means maternal urinary triclosan levels at any of the four study visits ($F=1.01$, $p=0.52$). Results are reported in Figure 13.

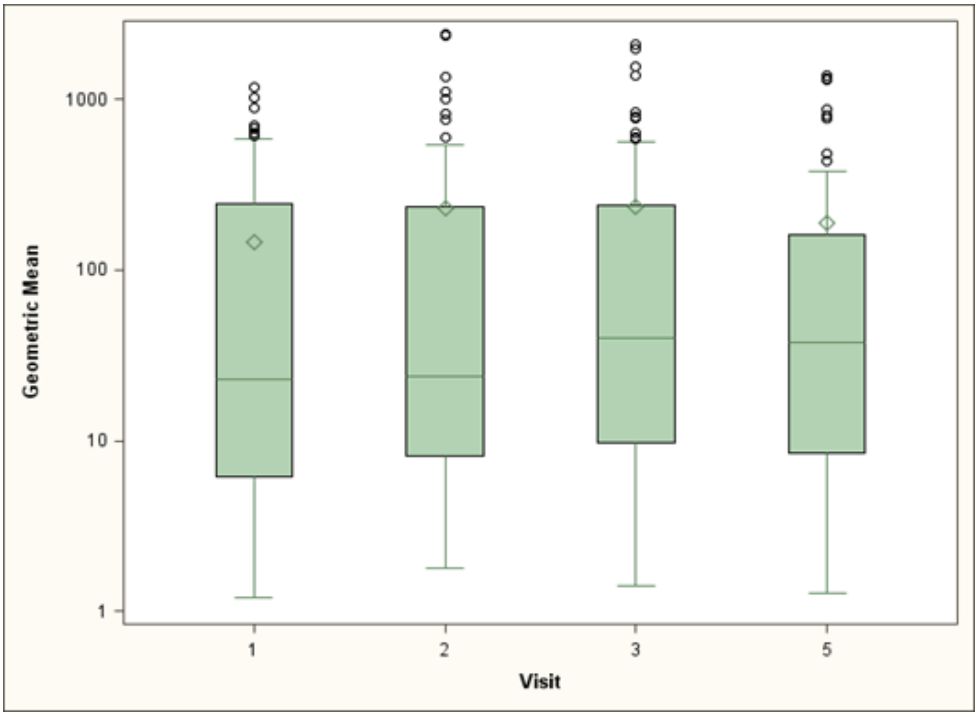


*Figure 13.*Boxplot of the specific gravity adjusted geometric means (µg/L) of all individual maternal urine samples by study visit. Y-axis log log base 10 scale.

5.2.3.2 Objective 3b: To evaluate the ability of a single spot urine sample to correctly predict an individual's level of exposure to triclosan.

Terciles (low, medium, and high exposure) were identified based on the distribution of all individual specific-gravity adjusted geometric means from the T1A and T1B study visits. Geometric means of 0µg/L to less than 11.49µg/L were classified as low exposure. Those between 11.49µg/L and under 136.67µg/L fell into the medium exposure category. Geometric mean triclosan levels of 136.67µg/L or greater were classified as high exposure.

The overall accuracy of a single spot sample collected in visit T1A or T1B to correctly predict an individual's overall exposure to triclosan was 86.7%. The prediction accuracy was lower for women who had geometric mean triclosan levels in the medium

exposure tercile (79% accuracy) compared to low (91% accuracy) and high exposure terciles (91% accuracy) (χ^2=25.66, p<0.0001).

When comparing the ability of a single spot sample collected on a week-day versus a week-end day to correctly predict an individual's overall exposure to triclosan, accuracy was highest among week-day samples (χ^2=11.28, p=0.0008). Week-day samples were accurate 90.3% of the time, while week-end day samples were accurate 83.2% of the time. For both week-day and week-end day urine samples, the prediction accuracy was lowest for geometric mean TCS levels in the medium terciles (T1A: χ^2=11.01, p=0.0041; T1B: χ^2=17.93, p=0.0001). These results are illustrated in Tables 7 and 8.

Table 7. Prediction accuracy of a week-day single spot sample to correctly identify an individual's overall geometric mean triclosan level.

	Low Tercile	Medium Tercile	High Tercile	Total
Correct Prediction	159	142	156	457
Total	166	167	173	506
Percentage	95.8%	85.0%	90.2%	90.3%

Table 8. Prediction accuracy of a week-end day single spot sample to correctly identify an individual's overall geometric mean triclosan level.

	Low Tercile	Medium Tercile	High Tercile	Total
Correct Prediction	145	144	136	425
Total	169	193	149	511
Percentage	95.8%	85.0%	90.2%	83.2%

Prediction of overall geometric mean triclosan levels was equally accurate among urine samples provided at various time points throughout the day (χ^2=2.51, p=0.28); however, single spot samples collected between 09:00 and 15:59 were more accurate at predicting high urinary triclosan levels (χ^2=15.52, p=0.0004). Results are detailed in Table 9.

Table 9. Prediction accuracy of high urinary triclosan levels by time of day of urine sample collection.

	00:00-08:59	09:00-15:59	16:00-23:59	Total
Correct Prediction	59	135	98	292
Total	67	138	117	322
Percentage	88.1%	97.8%	83.8%	90.7%

5.2.4 Objective 4: To determine the pattern of urinary triclosan concentrations in a 48-hour time period following triclosan exposure.

There was a high amount of variability in the urinary triclosan levels following exposure to a product containing triclosan. Individual profiles of study participants and the average trend line are plotted in Figure 14. The resulting trend line demonstrated that time should be modelled cubicly; however, due to the heterogeneity of the data and the limited sample size, a parametric linear trend model was judged as an adequate model for the study data.

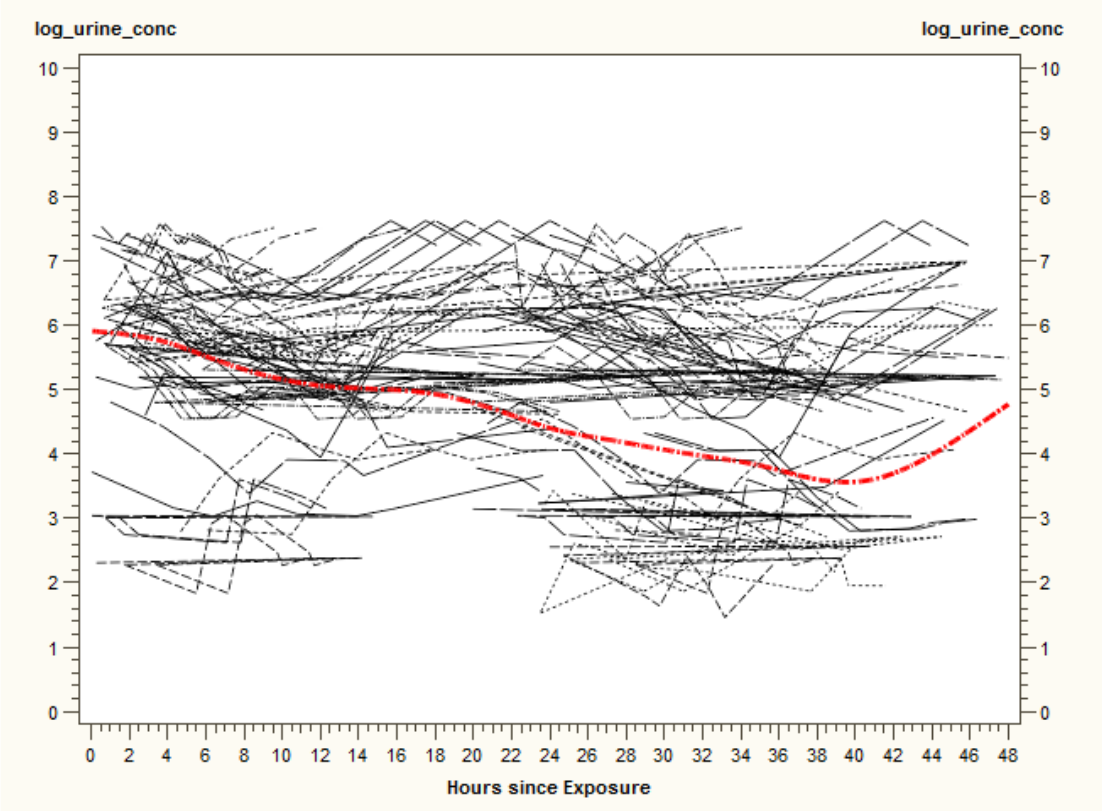


Figure 14. Individual profiles of log-transformed urinary triclosan concentration levels by time since exposure to a triclosan product in hours.

Following exposure to a product containing triclosan, the urinary triclosan concentration dropped at a rate of 0.4% per hour (t=-2.31, p=0.022). This is graphically displayed in Figure 15.

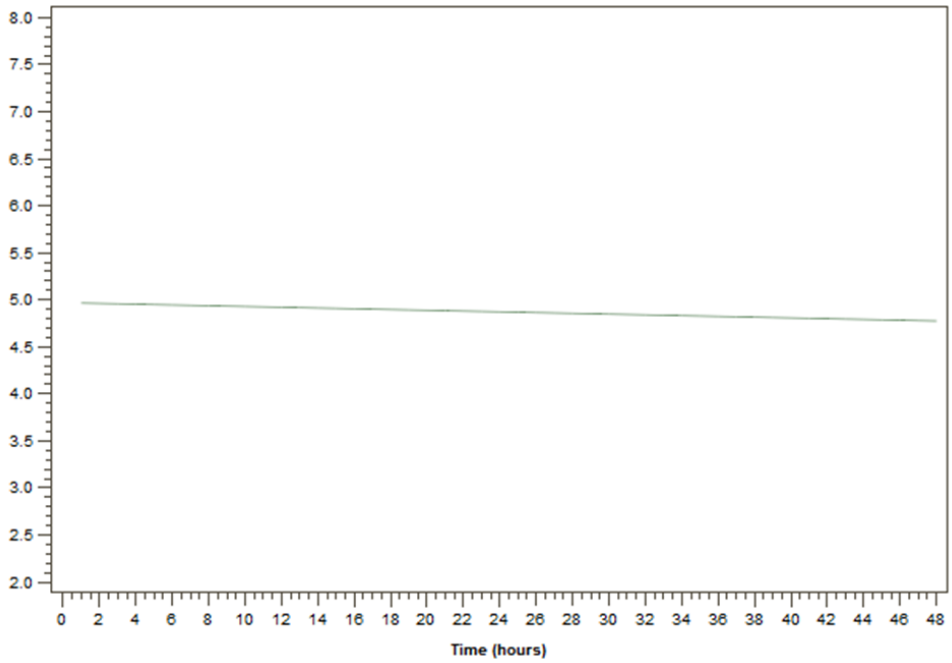


Figure 15. Parametric linear trend model of log-urinary triclosan concentration by time since exposure to a triclosan product across a 48-hour time period.

5.3 SUMMARY OF FINDINGS

The study sample included 80 pregnant women, with an average maternal age of 32.8 years (standard deviation=4.70).

There were a total of 6031 recorded products used across pregnancy; hand soaps/sanitizers (15.0%) and lotions/creams (14.5%) were most frequently used by the study participants (Figure 5). Of the 6031 total product uses, 266 (4.41%) contained triclosan (Figure 6). Those products containing triclosan were primary oral care products (46.6%) and hand soaps/sanitizers (42.4%) (Figure 7).

Linear mixed effects modeling identified that time of day, season of urine sampling, and parity were significant predictors of urinary triclosan levels (Table 5). Specifically, the geometric means of urine samples provided between 16:00 and 23:59 were significantly lower than those provided from 00:00 to 08:00 or from 09:00 to 15:59. The geometric mean

of urine samples collected in autumn was 1.44 times higher than those collected in winter. Women with no previous pregnancies had geometric mean urinary triclosan levels 6.04 times greater than those with 2 or more previous pregnancies.

A total of 1247 urine samples were provided among study participants. Adjusting for specific-gravity, 86.8% of these samples had detectable levels of triclosan. Triclosan was detected in the urine of 51.25% of the pregnant women. The geometric mean specific-gravity adjusted triclosan level of all the urine samples was 34.1µg/L (30.5-38.0) (Table 6). An intra-class correlation coefficient of 0.24 indicated poor reproducibility among urine samples collected across pregnancy (Figure 11). Geometric mean specific-gravity adjusted urinary triclosan levels were not different between week-day and week-end day samples (χ^2=0.0026, p=0.96) (Figure 12), nor between samples collected at different study visits (F=1.01, p=0.52) (Figure 13).

When using a single spot urine sample to predict an individual's overall geometric mean urinary triclosan level corresponding to low, medium, or high exposure, the overall accuracy was 86.7%. Single spot urine samples had significantly lower accuracy when predicting exposure into a medium exposure category (χ^2=25.66, p<0.0001). Accuracy was significantly higher among samples collected on a week-day when compared to those collected on a week-end day (χ^2=11.28, p=0.0008) (Table 7 and 8). When assessing the accuracy of a single spot sample to predict geometric mean urinary triclosan levels representing high exposure, samples collected between 09:00 and 15:59 (χ^2=15.52, p=0.0004) had significantly higher accuracy (Table 9).

Following exposure to a product containing triclosan, urinary triclosan levels significantly decreased at a linear rate of 0.4% per hour (t=-2.31, p=0.022) (Figure 15).

CHAPTER 6: DISCUSSION

The present study provided the first Canadian data on personal care product use and urinary maternal triclosan levels throughout pregnancy. The study findings indicated that pregnant women were exposed to triclosan through numerous product sources; the detection of maternal urinary triclosan levels persisted throughout the duration of the pregnancy as well as postpartum.

6.1 EXPLANATIONS FOR THE STUDY FINDINGS

6.1.1 Sample characteristics

As the General Campus of the Ottawa Hospital is a tertiary care hospital, some of the pool of potential participants from that site may have been high risk pregnancies; however, low risk pregnancies were also seen at the General Campus. Other recruitment sites such as the Harmony obstetrical clinic would also be less likely to recruit participants with high risk pregnancies, thereby creating the most representative sample possible.

While attempts to recruit participants from all socio-economic strata were made, the P4 study population was a highly educated sample; 45% had an Undergraduate University Degree and 26% had a Masters or a Doctorate degree. For this reason, these women may have had different personal care product use patterns or lifestyle changes throughout their pregnancy compared to those with less education or income and the results of the study are less generalizable.

A higher percentage of highly educated participants was also observed in several other studies, including Braun and colleagues (2012) , who measured the temporal variability of bisphenol A, (also a phenol like triclosan), in a United States pregnant population.

Woodruff and colleagues (2011) measured urinary triclosan levels in a United States general population; 59% of their study population were in the highest categorized education category (greater than high school diploma). In the study by Meeker and colleagues (2013) on Puerto Rican pregnant women, 82% of the study population had a college degree, which underlies the difficulties in obtaining a representative population of pregnant women across socio-economic lines in longitudinal follow-up biomonitoring studies. The increased education level of the P4 study participants could have resulted in a heightened awareness of antimicrobials, leading to an active avoidance of specific antimicrobial products. Women were not asked whether or not they were actively trying to avoid the use of antimicrobial products. The 4.41% of products used containing triclosan may be an underestimation due to the nature of the study population and the means of collecting the data. It is difficult to get women to accurately and completely record all products used.

6.1.2 Personal care product use

All data collected in this study pertaining to product use information were self-reported. The information collected was only as accurate as the information provided by the study participants. Missing product use information was high for several reasons: 19 women lost their "Product Use Booklet" throughout the duration of the study; information could not be collected from participants with early outcomes or who were lost to follow up over the year; "Food and Activity Diary" and "Product Use Booklet" information did not always match up; insufficient product information such as brand name and/or manufacturer of the product resulted in an inability to properly categorize the product. Overall, approximately 25% of all recorded product uses had insufficient information to properly identify whether or not triclosan was an active ingredient in the product. More detailed product information was expected among participants; the high number of insufficient information provided was

disappointing. The high percentage of products with insufficient information provided provides evidence towards supporting the conclusion that the actual number of triclosan products used may be greater than 5%. The only other previous study to date measuring triclosan product use did not require participants to record product brand and manufacturer information, in hopes of retaining power to test for associations (Meeker et al. 2013). Although the P4 study did lose power by collecting this information, it is important knowledge to understand the details of triclosan product sources that may be associated with increased urinary triclosan levels.

The findings of this study indicated that 89% of all triclosan products used by the pregnant women were "oral care" products or "hand soaps/sanitizers". These findings are slightly different from the only previous study to identify triclosan product uses. Meeker and colleagues (2013) also identified "hand soaps/sanitizers" as a commonly used product categories throughout their study population of Puerto Rican women; however, their research study failed to include "oral care" as a category. Rather, they found a high number of "hair care" product uses, due to the use of hairspray. The P4 study findings did not indicate any association between "hair care" products and urinary triclosan levels. This difference in study findings could be attributable to cultural differences between Canadian and Puerto Rican women and the products available in the two countries.

It was hypothesized that pregnant women would be exposed to triclosan through cosmetics, soaps, and toothpaste. Soaps and toothpaste were in fact common sources of exposure. Despite the fact that cosmetics were a commonly used product, they were not found to be a statistically significant product source of triclosan in this population.

There was a statistically significant positive correlation between urinary triclosan concentration and triclosan product use. In a study of bisphenol A sublingual exposure by

Gayrard and colleagues (2013), sublingual absorption was higher than the gastro-intestinal absorption from the oral dosing (Gayrard et al. 2013). “Oral care” products and “hand soaps/sanitizers” were the most commonly used products containing triclosan among the study population. Based on the high sublingual absorption rates of phenols, it is plausible that an increased concentration of urinary triclosan levels could be observed in this study population due to the “oral care” products. However, the geometric mean of specific gravity adjusted triclosan levels was no different between the “oral care” (79.92 µg/L (95% CI 59.29-107.73)) and the “hand soaps/sanitizer” (80.73 µg/L (95% CI 63.37-102.85)) groups.

6.1.3 Covariate associations with urinary triclosan levels

The P4 Study findings indicated that triclosan levels were significantly lower among samples collected between 16:00 and 23:59. This is consistent with previous literature on the temporal variability of phenols reporting the lowest phenol levels after 16:00 (Mahalingaiah et al. 2008), and confirm the hypothesis that time of day of sample collection would predict urinary triclosan levels.

Triclosan levels were significantly higher among urine samples collected in autumn when compared to those collected in winter. This could be the result of product use differences between the two seasons. With the colder winter weather, it is possible that the women did not leave the house as much, and therefore did not use as many products containing triclosan. Although no statistically significant differences were observed between urine samples collected in autumn and those collected in spring or summer, it is possible that the small sample size of this study did not produce enough power to detect these differences.

Overall, parity was a significant predictor of urinary triclosan levels. Pairwise comparisons concluded that triclosan levels in women with no previous pregnancies were significantly higher than samples collected from women with two of more previous

pregnancies. This could be due to the fact that they had less time to devote to personal care product use because of the need to care for their children. Again, with a larger sample size, a difference in triclosan levels between women with no previous pregnancies and those with 1 previous pregnancy may have been observed.

Education and combined household income were not statistically significant predictors of urinary triclosan levels in any of the three models. This is in contrast with previous studies which have reported differing conclusions regarding associations between education and household income on urinary triclosan levels. Specifically, an inverse relationship was noted between education level and phenol concentration (Wolff et al. 2008), while a positive association (Ye et al. 2008) and an inverse association (Kim et al. 2011) were reported between household income and phenol concentration. Inconsistent results have also been reported on maternal age; the results of the P4 Study did not confirm any of these associations; however, it is possible that with a larger sample size, an association may have been observed.

Model 1, which did not include specific gravity at all in the model, reported that total volume and time since last void were statistically significant predictors of the urinary triclosan levels. Total volume and time since last void were not statistically significant in Model 2 or Model 3, which included it as a covariate and adjusted for specific gravity in the dependent variable, respectively. This could indicate that total volume and time since last void are less important to control for if the specific gravity measurement is known.

6.1.4 Urinary metabolite levels

Missed urine samples were also reflected in the number of urine samples provided by participants across pregnancy; the minimum number of samples provided was 4, which is highly unlikely given the serial urine sample collection periods in early pregnancy. Study

nurses reminded participants about each upcoming study visit; however, many samples were still unrecorded despite these reminders.

Despite the fact that 49.75% of the study sample did not have any detectable levels of triclosan in their urine samples, 86.8% of all the urine samples still contained levels of triclosan greater than the limit of detection (3.0µg/L). This demonstrates that the women with detectable levels of triclosan contributed an overall larger number of urine samples than those with triclosan levels consistently below the limit of detection.

Usually laboratories do not release biomonitoring data below the limit of detection (LOD). The LOD is the lowest quantity of a substance that can be distinguished from the absence of that substance (a *blank value*) within a stated confidence limit (McNaught and Wilkinson 1997). In this study, the laboratory was asked to report urinary triclosan levels regardless of their relation to the limit of detection. As a result, measurements between 0 and 3.0µg/L were reported, thereby reducing potential biases that can be affected by using a constant such as one-half the limit of detection.

The unadjusted maternal urinary triclosan concentrations reported in this study were nearly twice as high as many previously reported levels. The observed geometric mean urinary triclosan levels among P4 Study participants was 32.1µg/L (28.7-36.1), which was twice as high as levels found in two previous Canadian studies. The 2013 Canadian Health Measures Survey data reported a geometric mean of only 16.0µg/L (Health Canada 2013b) while the 2012 report published provided by Environmental Defence reported an arithmetic mean of 15.48µg/L (Environmental Defence 2012). It is important to note that the Canadian Health Measures Survey samples were also analysed by the same Quebec laboratory using the same methods as in the P4 Study. In two studies of United States pregnant populations, geometric mean urinary triclosan levels were 17.00µg/L and 13.0µg/L, respectively (Calafat

et al. 2008; Woodruff et al. 2011). The difference between the P4 Study findings and those of the United States studies could be attributable to a difference in product availability of certain triclosan products between the two countries; however, the urine samples for these studies were collected between 2003 and 2004, 6 to 7 years prior to that of the P4 Study (2009 to 2010 collection period). The urine samples collected from the Canadian Health Measures Survey were collected between 2009 and 2011, which is a comparable collection time period to that of the P4 Study. Further Canadian research is required to confirm these elevated urinary triclosan measurements in pregnant women and then attempt to understand why they might be higher in this population.

Although differences were observed between the P4 Study findings and those of previous studies, the most recently published study on maternal urinary triclosan levels by Meeker and colleagues (2013) reported a geometric mean urinary triclosan level of 29.9 (23.6-37.9), which is very comparable to the results of the P4 Study. The similarities in geometric mean urinary triclosan levels could be attributable to a similar urine collection schedule between the two studies. Urine samples were collected at multiple times across pregnancy, rather than once in the third trimester, as was done in previous studies (Calafat et al. 2008; Woodruff et al. 2011).

The calculation performed for the 95% confidence intervals of urinary triclosan level percentiles in the P4 Study assumed that the data are normally distributed. The urinary triclosan levels were not normally distributed, as was reflected by the log transformation of the data for the linear mixed effect models. In order to account for this, the geometric mean and median were reported in Table 6.

The P4 Study data reported some extreme urinary triclosan measurements, including a maximum unadjusted urinary triclosan measurement of 3229.3µg/L. Qualitative

employment information on selected participants recording extreme urinary triclosan measurements does not show a distinct pattern; however, approximately two-thirds of participants reporting outlying levels worked in a government office or in a hospital (Table 10). These participants did not report repeated outlying urinary triclosan levels throughout the study. Therefore, factors leading to these high outliers remain unknown. It is unlikely that these results are due to lab error, as extreme urinary triclosan measurements of 2000µg/L (Meeker et al., 2013), 3157.7µg/L (Kim et al., 2011) and >6000µg/L (Tye Arbuckle, unpublished data, 2013) have also been reported in urinary triclosan studies of pregnant populations.

Table 10. Qualitative data on occupation of nine P4 study participants who recorded high outlier urinary triclosan levels.

Unadjusted urinary TCS (µg/L)	Occupation
3229.26	Government
2803.41	Government
2629.95	Construction Engineer
2550.73	Physician
2528.34	Unemployed
2216.43	Esthetician
2136.98	Unemployed
2037.88	Government
1269.23	Registered Nurse

The intra-class correlation coefficient of the samples collected throughout the course of the P4 Study was 0.24, which indicated poor reproducibility among samples collected at different time points across pregnancy. This result showed a lower level of reproducibility than that of Meeker and colleagues (2013), who reported a specific-gravity adjusted intra-class correlation coefficient of 0.47 among single spot urine samples collected 3 times during gestation. The lower ICC reported in the P4 Study could have been a result of the high level of variability in the data. In addition to measuring urinary triclosan levels across pregnancy, the P4 Study also collected serial urine samples on both a week-day and a week-end day during the first 20 weeks of pregnancy. Thus, it was possible to conclude that the urinary triclosan levels were no different between samples collected on a week-day versus those collected on a week-end day.

Urinary triclosan levels were lower from 16:00-23:59 when compared to 00:00-08:59 and to 09:00-15:59. It is possible that this difference is related to the timing of triclosan product use. Table 11a indicates the percentage of product use by the time of day categories used in the linear mixed effects model analysis, while Table 11b further breaks down the time of day in order to obtain increased detail pertaining to the timing of the triclosan product use among study participants.

Table 11a. Triclosan product uses by time of day (3 categories).

Time of Day	N	Percentage
00:00-08:59	39	14.7%
09:00-14:59	127	47.7%
15:00-23:59	97	36.5%
Missing	3	1.1%

Table 11b. Triclosan product uses by time of day (6 categories).

Time of Day	N	Percentage
00:00-03:59	21	7.9%
04:00-07:59	18	6.8%
08:00-11:59	25	9.4%
12:00-15:59	102	38.3%
16:00-19:59	68	25.6%
20:00-23:59	29	10.9%
Missing	3	1.1%

Approximately 85% of all triclosan product uses occurred between 09:00 and 23:59. Specifically, 63.9% of all recorded products used were between 12:00 and 19:59. These results indicate that triclosan product exposure is less common through products used in the morning; rather, product use throughout the day and early evening is more common. As the urinary half-life of triclosan is suggested to be approximately 11 hours, the low urinary triclosan levels from 15:59-23:59 could be a reflection of the low percentage of triclosan product use in the early morning. These findings confirm the hypothesis that differences in the timing of product exposure result in individual differences in urinary triclosan levels.

6.1.5 Predictive ability of single spot samples

The serial urine sample collection also allowed for calculation of the predictive ability of single spot samples collected at various time points throughout a day, as well as on a week-day and on a week-end day. Although collecting serial urine samples at each study

visit could increase the precision and power of the results, it remains cost-effective to limit the number of serial urine samples collected in a study. Based on the findings of the P4 Study that there was no statistically significant difference in the geometric mean urinary triclosan levels across the study visits, the results obtained from the serial urine samples can be extrapolated to the subsequent study visits. Future research could collect serial urine samples in the second or third trimester and compare the results of a surrogate category analysis with the early pregnancy results from the P4 Study.

The surrogate category analysis indicated that the accuracy of single spot urine samples was lower when identifying individuals with moderate urinary metabolite levels. From a risk assessment perspective, it is most important to identify individuals with high levels of urinary triclosan. Single spot urine samples consistently predicted high metabolite levels more than 90% of the time, which is consistent with the hypothesis stating that a single spot sample would be a reliable indicator of an individual's average exposure.

Increased precision of the prediction ability of a single spot sample could have been achieved if the time of day had been categorized into more than three categories. Due to the small sample size in this study, the number of urine samples per cell became insufficient as the 24-day was broken down into a greater number of categories.

6.1.6 Parametric linear trend model

Although the parametric linear trend model possessed the ability to identify the rate of linear decrease of urinary triclosan concentrations following exposure to a triclosan product, it remains hypothetical. An assumption of this model was that an individual had only one exposure to triclosan within a 48-hour time period. A future model should be developed that accounts for the multiple exposures to triclosan within a 48-hour time period.

Another possible way to model the trend of urinary triclosan concentration following exposure to triclosan would be through the use of a spline model. This model would allow for the 48-hour period to be broken down into smaller time periods; the pattern of triclosan would be modelled within each of these. Due to the small sample size, there was a large amount of variability in the P4 Study data. This variability could be due to the route of exposure, the concentration of triclosan in the product, and the ease of exposure to triclosan upon use of a triclosan containing product. As a result, the selection of the number and location of the knots corresponding to the multiple time periods was highly influential on the outcome of the model. For this reason, it was not possible to determine an optimal location and number of knots, and the parametric linear trend model was the chosen model.

Because of the selection of a parametric linear trend model, it was not possible to confirm the hypothesis predicting the pattern of urinary triclosan levels following exposure to a product containing triclosan. In a previous paper by Meeker et al. (2012) on urinary phthalate metabolites, the timing since last exposure was said to be an important contributor to the phthalate concentrations (Meeker et al. 2012). Future research is required to determine the pattern of urinary triclosan levels following exposure to assess the relationship.

6.2 LIMITATIONS

The repeated measures and longitudinal nature of this study design was susceptible to many limitations, particularly through the selection of subjects, as well as the self-reporting of activity and product use information.

Loss to follow-up:

Loss to follow-up or participant drop out was a limitation of this longitudinal study design. Throughout the study period, participants may have relocated, changed their contact

information or chosen not to continue with the study for various reasons. To address this limitation, a power calculation was performed to identify the minimum number of participants required to obtain the desired level of power for the study analyses and additional women were recruited to compensate for potential loss to follow-up.

Power:

The proposed sample size of 80 women was sufficient to estimate the variability within each individual with an error of less than 0.06 (power=0.80). However, the sample size proved to not be large enough to detect many statistically significant differences. Because of small and sometimes zero cell counts (for example, in the time of day of urine sampling variable) it was not possible to categorize certain variables into a larger number of categories. A larger sample size would have increased precision of the reported results and could have resulted in additional power to support the study findings.

Convenience sample:

The sample for the P4 Study was a convenience sample. Given that only approximately 20% of all women in the sampling frame were approached to take part in the study, this largely influences the generalizability of the study results. Women who were informed of P4 Study were only those who attended their ultrasound within the specified eligible gestation time, were those with high-risk pregnancies, or were those who visited the hospital at a time when a study researcher was working. Only two researchers were responsible for recruitment; each one was responsible for balancing the P4 Study recruitment with their full-time jobs. This resulted in convenience sampling, and many potentially eligible and interested participants never being invited to participate.

Selection bias:

Selection bias occurred in the study. Study participants were informed prior to enrolling in the study that one of the measured study chemicals was an antimicrobial. It is possible that the women who volunteered to enroll in the study were more knowledgeable and interested in this topic and consequently used less products containing triclosan, an antimicrobial agent. Another possibility is that the women who volunteered to participate in this research study may have had lifestyles that allowed time for record keeping and urine collections throughout the duration of the study. This may have led to biased results; reported triclosan product use may have been under-reported due to the associated selection bias. However, the higher geometric mean urinary triclosan concentrations would argue against less use of antimicrobial products containing triclosan.

Although it was not possible to overcome this limitation, controlling for covariates in the study limited the extent of selection bias on the study population. While the study results are not generalizable to the Canadian population, this should not have affected the internal validity of the study.

Interviewer bias:

Interviewer bias may occur if the interviewer tends to reveal more study information or selectively recruit a certain group of individuals. Use of a standardized data collection form was used to minimize interviewer bias.

Missing data/Recall bias:

Studies requiring participants to self-report data are more likely to encounter missing data for various reasons. It would have been easy for study participants to not prioritize the study diary and booklet completion. As a result, when they would have remembered to fill in their activities and product use information, certain activities, products, and details may have been forgotten.

Self-completion of questionnaires is also very time-consuming. For this reason, the degree of detail in the thoroughness of completion of diaries and booklets may have highly differed between participants.

Another reason for missing data is simply no reporting at all due to the loss of questionnaire material by the study participant. The repeated visits of this study were a way to remind participants about their diary completion; however, the final self-reported data was only as accurate as the information that was provided by the study participants regardless of the efforts undertaken to increase reporting.

CHAPTER 7: SUMMARY CONCLUSION

This study set out to address the existing knowledge gaps pertaining to urinary triclosan data in Canadian pregnant women. It was the first of its kind to provide Canadian data on personal care product use and urinary maternal triclosan levels within a day, a week-day and week-end day, and throughout pregnancy. It also was the first to provide information on the predictive ability of a single spot urine sample in pregnancy and to develop a linear trend model to represent the urinary triclosan concentration following exposure to a product containing triclosan. Given that the study sample is poorly representative of the Ottawa or Canadian population, the study results are not generalizable; however, they provide important information towards understanding sources of exposure to triclosan and towards characterizing the nature of the exposure risk, if any, associated with triclosan.

The findings indicate that the urinary triclosan levels of our study sample of pregnant women are consistent across pregnancy; levels are lowest between 16:00 and 23:59. Single spot urine samples accurately predict an individual's overall exposure 87% of the time; accuracy is highest among single spot urine samples collected between 09:00 and 16:00. The study participants are primarily exposed to triclosan through the use of "oral care" products and "hand soaps/sanitizers" containing triclosan. There is a positive association between triclosan product use and urinary triclosan levels. Following exposure to a product containing triclosan, there is a statistically significant linear decrease in urinary triclosan levels at a rate of 0.004μg/L per hour. Highly educated pregnant women may also be more highly exposed to triclosan than the general population, given the results of our study.

The results of this study can provide important information to policy makers, researchers and consumers. Policy makers and researchers can use this knowledge for future risk assessment and risk management. On a different level, it is important for consumers to

understand sources of exposure to triclosan and the basic pharmacokinetics of urinary excretion of triclosan in order to make personal choices regarding purchase of personal care products. The knowledge of current exposure levels and the identification of triclosan product sources associated with these levels can also assist with population-specific exposure assessment strategies in Canadian populations, as well as to ongoing assessment studies worldwide.

Additional studies are required to further examine the associations between specific covariates and urinary triclosan levels, which could direct the focus of educational awareness of antimicrobial agents. Expansion on the model of urinary triclosan levels following multiple exposures to triclosan is required. Further studies can replicate the methods of this study design and analysis to test hypotheses in different sub-populations.

Clearly the information on current Canadian maternal triclosan levels, sources of exposure, and the predictive ability of single spot urine sampling, as well as the basic pharmacokinetics of triclosan in combination with other knowledge is of great value for policy makers, consumers, risk assessors, and health care providers, among others. The information provided from this study can be readily used to further direct exposure controls and risk management procedures.

APPENDIX A – ENVIRONMENTAL DEFENCE REPORT

ENVIRONMENTAL DEFENCE REPORT, MAY 16TH 2012
May 16 2012
Canada NewsWire
First-of-kind data find toxic chemical widespread in Canadian adults

TORONTO, May 16, 2012 /CNW/ - The first data on levels of the chemical triclosan were released today to reveal widespread body pollution in Canadian adults. Seven of eight people tested had the antibacterial chemical in their bodies-with levels higher than those toxic to fish and algae.

Environmental Defence, which conducted the test, says the high prevalence of the chemical means it's time to ban it from household use. This backs up an earlier call from Canadian Medical Association, due to fears its widespread use contributes to antibiotic-resistant "superbugs."

Triclosan is an anti-bacterial chemical originally used in medical settings. But now it is found in hundreds of products, including hand sanitizers, toothpaste, household items, makeup and even smartphone cases. This is worrisome given it is also a known endocrine disruptor-interfering with the human body's natural hormones. Many endocrine disruptors have been linked to thyroid problems and cancer.

The Environmental Defence report The Trouble with Triclosan can be downloaded at www.environmentaldefence.ca/troublewithtriclosan

"Mounting evidence has convinced doctors and scientists that this chemical is, in fact, harmful and should be banned from household use," said Dr.Rick Smith, executive director of Environmental Defence and co-author of the bestselling book on toxic products, Slow Death by Rubber Duck. "Today's data show how widespread the chemical is in our bodies. So consumers should do what they can to avoid products that contain it. Because the danger with triclosan isn't just the level of exposure, it's also the length of time someone is exposed."

“Environmental Defence believes that a ban on the household use of triclosan is good for human health and our environment. We congratulate the federal government on its first steps in dealing with the chemical, and look forward to the next ones," he said.

On March 30, Health Canada and Environment Canada published a preliminary assessment of triclosan on the Chemical Substances website, commencing a 60-day public comment period. The assessment declared that found it can cause harm to the environment.

Environmental Defence has long called for better controls on toxic chemicals such as triclosan, and has successfully advocated to ban BPA in baby bottles and phthalates in toys.

Now it's turning its attention to triclosan, which contaminates the environment as it is washed down drains to pollute rivers and lakes. It is toxic to fish, amphibians and rats, where

it mimics thyroid hormones. This raises questions about its harm to human health. In addition, when it breaks down, it can produce the human carcinogens chloroform, and dioxins, one of the most toxic groups of substances known.

"Every time we wash our hands or brush our teeth with triclosan, more of this hormone-disrupting chemical goes down the drain. That's bad news for people and bad news for the environment and why it's time for a household ban," said Smith.

About Environmental Defence (www.environmentaldefence.ca): Environmental Defence is Canada's most effective environmental action organization. We challenge, and inspire change in government, business and people to ensure a greener, healthier and prosperous life for all.

APPENDIX B – BIOSPECIMEN TRACKING LOGS

The P4 Study: Biospecimen Tracking Log: T1a Maternal Urine

Date Samples Received: (in lab) Day / Month / Year **Time** : (24 hr clock) **Participant ID** **Lab Personnel's Initials** **Freezing Time** : (24 hr clock)

Collection Container					INSPQ Aliquots						Biobank Aliquots							
120 mL Nalgene	Date of Collection dd/mmm/yy	Time of collection	Total Volume	Specific Gravity	30 mL Nalgene	Volume Aliquotted	30 mL Nalgene	Volume Aliquotted	30 mL Nalgene	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted
Preferred Vol (mLs)					Phthalates	10	BPA, TCS TCC	10	Naph, cotinine	5	Biobank	5	Biobank	5	Biobank	5	Biobank	5
T1a.1					T1a.1.1		T1a.1.2				T1a.1.3		T1a.1.4		T1a.1.5		T1a.1.6	
T1a.2					T1a.2.1		T1a.2.2				T1a.2.3		T1a.2.4		T1a.2.5		T1a.2.6	
T1a.3					T1a.3.1		T1a.3.2				T1a.3.3		T1a.3.4		T1a.3.5		T1a.3.6	
T1a.4					T1a.4.1		T1a.4.2				T1a.4.3		T1a.4.4		T1a.4.5		T1a.4.6	
T1a.5					T1a.5.1		T1a.5.2				T1a.5.3		T1a.5.4		T1a.5.5		T1a.5.6	
T1a.6					T1a.6.1		T1a.6.2				T1a.6.3		T1a.6.4		T1a.6.5		T1a.6.6	
T1a.7					T1a.7.1		T1a.7.2				T1a.7.3		T1a.7.4		T1a.7.5		T1a.7.6	
T1a.8					T1a.8.1		T1a.8.2				T1a.8.3		T1a.8.4		T1a.8.5		T1a.8.6	
T1a.9					T1a.9.1		T1a.9.2				T1a.9.3		T1a.9.4		T1a.9.5		T1a.9.6	
T1a.10					T1a.10.1		T1a.10.2				T1a.10.3		T1a.10.4		T1a.10.5		T1a.10.6	
T1a.P									T1a.P									
T1a. FB					T1a.FB.1		T1a.FB.2				T1a.FB.3		T1a.FB.4		T1a.FB.5		T1a.FB.6	

Unused Biobank Aliquot Labels (one row per collection container)				Comments

Additional Comments:

_____
_____
_____
_____

The P4 Study: Biospecimen Tracking Log: T1b Maternal Urine

Date Samples Received: ____ ____ ____ **Time** ____ : ____ ____ ____ ____ ____ **Freezing Time** ____ : ____
(in lab) **Day / Month / Year** **(24 hr clock)** **Participant ID** **Lab Personnel's Initials** **(24 hr clock)**

Collection Container					INSPQ Aliquots						Biobank Aliquots							
120mL Nalgene	Date of Collection dd/mmm/yy	Time collection (24 hr)	Total Volume	Specific Gravity	30 mL Nalgene	Volume Aliquotted	30 mL Nalgene	Volume Aliquotted	30 mL Nalgene	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted
Preferred Volume (mLs)					Phthalates	10	BPA, TCS TCC	10	Naph, cotinine	5	Biobank	5	Biobank	5	Biobank	5	Biobank	5
T1b.1					T1b.1.1		T1b.1.2				T1b.1.3		T1b.1.4		T1b.1.5		T1b.1.6	
T1b.2					T1b.2.1		T1b.2.2				T1b.2.3		T1b.2.4		T1b.2.5		T1b.2.6	
T1b.3					T1b.3.1		T1b.3.2				T1b.3.3		T1b.3.4		T1b.3.5		T1b.3.6	
T1b.4					T1b.4.1		T1b.4.2				T1b.4.3		T1b.4.4		T1b.4.5		T1b.4.6	
T1b.5					T1b.5.1		T1b.5.2				T1b.5.3		T1b.5.4		T1b.5.5		T1b.5.6	
T1b.6					T1b.6.1		T1b.6.2				T1b.6.3		T1b.6.4		T1b.6.5		T1b.6.6	
T1b.7					T1b.7.1		T1b.7.2				T1b.7.3		T1b.7.4		T1b.7.5		T1b.7.6	
T1b.8					T1b.8.1		T1b.8.2				T1b.8.3		T1b.8.4		T1b.8.5		T1b.8.6	
T1b.9					T1b.9.1		T1b.9.2				T1b.9.3		T1b.9.4		T1b.9.5		T1b.9.6	
T1b.10					T1b.10.1		T1b.10.2				T1b.10.3		T1b.10.4		T1b.10.5		T1b.10.6	
Pooled									T1b.P									
T1b. FB					T1b.FB.1		T1b.FB.2				T1b.FB.3		T1b.FB.4		T1b.FB.5		T1b.FB.6	

Unused Biobank Aliquot Labels (one row per collection container)				Comments

Additional Comments:

_____

_____

_____

_____

The P4 Study: Biospecimen Tracking Log: T3 Maternal Urine

Date Samples Received: (in lab) ____ / ____ / ____ Day / Month / Year

Time ____ : ____ (24 hr clock)

Participant ID ____ ____ ____

Lab Personnel's Initials ____ ____

Freezing Time ____ : ____ (24 hr clock)

Collection Container					INSPQ Aliquots				Biobank Aliquots							
120mL Nalgene	Date of collection	Time of Collection	Total Volume	Specific Gravity	30 mL Nalgene	Volume Aliquotted	30 mL Nalgene	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted
Preferred Volume (mLs)					Phthalates	10	BPA, TCS TCC	10	Biobank	5	Biobank	5	Biobank	5	Biobank	5
T3.1					T3.1.1		T3.1.2		T3.1.3		T3.1.4		T3.1.5		T3.1.6	

Unused Aliquot Labels				Comments

Additional Comments:

_____

_____

_____

_____

The P4 Study: Biospecimen Tracking Log: T5 Maternal Urine

Date Samples Received: (in lab) Day / Month / Year **Time** : (24 hr clock) **Participant ID** **Lab Personnel's Initials** **Freezing Time** : (24 hr clock)

Collection Container					INSPQ Aliquots						Biobank Aliquots							
120mL Nalgene	Date of collection	Time of Collection	Total Volume	Specific Gravity	30 mL Nalgene	Volume Aliquotted	30 mL Nalgene	Volume Aliquotted	30 mL Nalgene	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted	5 mL Simport	Volume Aliquotted
Preferred Vol (mLs)					Phthalates	10	BPA, TCS TCC	10	Naph, Cotinine	5	Biobank	5	Biobank	5	Biobank	5	Biobank	5
T5.1					T5.1.1		T5.1.2		T5.1.3		T5.1.4		T5.1.5		T5.1.6		T5.1.7	

Unused Aliquot Labels				Comments

Comments:

_____

_____

_____

_____

APPENDIX C – STUDY RECRUITMENT POSTER

The P4 Study:
Plastics and Personal-care Product Use in Pregnancy

Are you between 6 and 19 weeks pregnant?

We are looking for participants for a new research study that will measure the exposure of pregnant women and their babies to chemicals found in various personal-care and food packaging products.

For more information please contact the study nurse coordinator:

[redacted]

This research project has been approved by The Ottawa Hospital Research Ethics Board.

Health Canada Santé Canada

OHRI IRSO

OMNI RESEARCH GROUP

Utilisation de matières plastiques et de produits de soins personnels pendant la grossesse

Êtes-vous enceinte de 6 à 19 semaines?

Nous sommes à la recherche de participantes pour une nouvelle étude qui examinera le taux d'exposition des femmes enceintes et de leur bébé aux substances chimiques contenues dans divers produits de soins personnels et emballages alimentaires.

Pour obtenir de plus amples renseignements, communiquez avec l'infirmière coordonnatrice de l'étude, au [redacted]

Le projet de recherche est approuvé par le Conseil d'éthique en recherches de l'Hôpital d'Ottawa.

Santé Canada Health Canada

OHRI IRSO

OMNI RESEARCH GROUP

AN INSTITUTE OF • UN INSTITUT DE

APPENDIX D – INFORMED CONSENT

The Ottawa Hospital | L'Hôpital d'Ottawa

Plastics and Personal-care Product use in Pregnancy: The P4 Study

Consent Form

I have read this six-page Patient Information and Consent Form and I have had the opportunity to ask the study investigator or research nurse coordinator any questions I may have had about the study. My questions and/or concerns have been answered to my satisfaction and I agree to participate in this study. If I decide at a later time in the study that I would like to withdraw, I may do so at any time and my withdrawal will not affect the quality of care I receive now and in the future at The Ottawa Hospital.

A copy of this Information and Consent Form will be provided to me.

Unless I have provided specific consent or where the law requires or a court order has been obtained, my results will not be communicated to any third parties such as my employers, governmental organizations or insurance companies. This includes my spouse or other members of my family. Any results of tests performed in this study will not be communicated to my health care provider nor will they appear in my medical record.

If I wish to withdraw from the study or remove my coded data and coded biological specimens, I must contact Dr. Mark Walker at The Ottawa Hospital or a member of his research team and request that my data and specimens be destroyed.

I hereby consent to participate and to have any leftover samples stored for future research directly related to environmental contaminants.

_____ _____ _____
Name of Participant Signature Date

_____
Telephone number

_____ _____ _____
Investigator/Delegate Signature Date

APPENDIX E – PARTICIPANT ELIGIBILITY SCREENING

INCLUSION CRITERIA: If the woman answers yes to *ALL* of the inclusion criteria questions she may be eligible for the study.	**Yes**	**No**
1. Singleton and viable fetus		
2. The woman is at least 6 $^{0/7}$ weeks pregnant and is able to complete the T1 urine collections (week-day and week-end multiple samples) before 19^{6} weeks. NOTE: if early ultrasound and LMP dates are discordant ≤ 7 days, base GA estimate on LMP dates, if discordant > 7 days base on ultrasound.		
3. Age ≥ 18 years		
4. Speaks French or English		
5. Plans to deliver in the city of Ottawa, including all Ottawa hospitals or home births		
6. The woman is able to understand and sign a consent form.		

EXCLUSION CRITERIA: If the woman answers YES to ANY of the exclusion criteria below she is *NOT* eligible for the study.	**Yes**	**No**
1. Known major fetal abnormalities, including chromosomal anomalies		
2. Has one of the following conditions		
2.1 Renal disease with altered renal function (creatinine ≥ 2 times the upper limit of normal range value)		
2.2 Any collagen vascular disease (including lupus erythematosus, scleroderma)		
2.3. Active or chronic hepatitis		
2.4 Epilepsy		
2.5 Heart disease		
2.6 Serious pulmonary disease (COPD)		
2.7 Cancer		
2.8 Haematologic disorder		
2.9 Threatened abortion		
2.10 Thyroid disorder; including treated hypothyroidism		
2.11 Chronic hypertension (diagnosed prior to pregnancy)		
2.12 Diabetes (do not exclude if participant develops gestational diabetes after recruitment)		
2.13 Current Illicit Drug use		

APPENDIX F – DATA AND MATERNAL COLLECTION SCHEDULES

I. Data Collection Schedule

Sample	Pregnancy				Post Delivery	
	1st trimester (6 – 19 wks)		2nd trimester (24-28 wks)	3rd trimester (32-36 wks)	PP* Day 1-3	2 -3 months PP
	T1a	T1b	T2	T3	T4	T5
Questionnaire	●		●	●		●
Journal	●	●	●	●		●
Maternal Urine	●	●	●	●		●
Medical Chart	●		●	●	●	
Meconium					●	
Infant Urine**					●	●
Breastmilk						●
Formula						●
Personal and indoor air samples	●	●				●

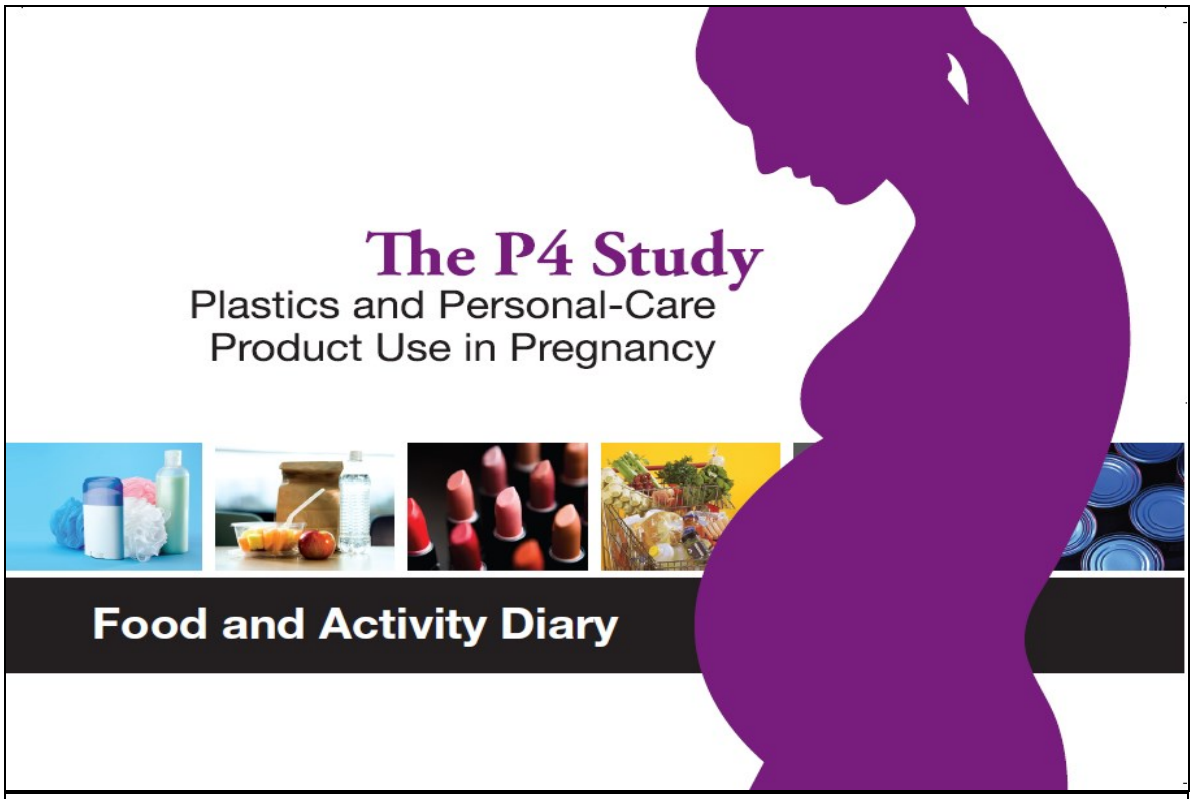
*PP=postpartum

**samples collected up to 1 month postpartum

II. Maternal Urine Collection

Sample Collection	1st trimester (6 – 19 wks)		2nd trimester (24-28 wks)	3rd trimester (32-36 wks)	2–3 mo PP
	T1a	T1b	T2	T3	T5
Day of the week	week-day	week-end	Either	Either	Either
Approx. # of samples	8	8	1	1	1
Type of sample	Multiple samples throughout a 24 hr period.	Multiple samples throughout a 24 hr period.	One spot urine sample	One spot urine sample	One spot urine sample

APPENDIX G – FOOD AND ACTIVITY DIARIES/PRODUCT USE BOOKLETS



Instructions for Food and Activity Diary

Why are we collecting this information?

Phthalates and Bisphenol A (BPA) are chemicals commonly used in consumer products and plastics such as food packaging, vinyl flooring, shampoo, cosmetics, canned food, and insect repellent. We are trying to understand what products women are using and how patterns of use change over a weekday versus a weekend. This will help us better understand how these products may contribute to everyday exposure.

Date and Time of Diary Started: YYYY / MM / DD _____ : _____ am / pm	
Study ID: _____	**Study Period:** _____

Date and time …	Year / Month / Day	Time
… of first urine sample?	_____ / _____ / _____	_____ : _____ am / pm
… since you last voided?	_____ / _____ / _____	_____ : _____ am / pm

When do I complete the Diary?

We would like you to record your use of consumer and personal care products and food packaging in the enclosed diary:

A. During your 1st trimester of pregnancy we will ask you to collect urine over two 24-hour periods (during a weekday and a weekend).

We ask that you complete the diary for:

- the 24-hour period before you start collecting your urine samples;
- the day of your 24-hour urine sample collections: a sample from all voids during the 24 hours;
- record the date and time of any missed urine collections.

	1st Trimester Samples			
	Weekdays		Weekend	
	Monday	Tuesday	Saturday	Sunday
Diary				
24-hour urine collection				

Example: If you started collecting your urine on Tuesday morning, you would complete a diary for Monday and Tuesday.

B. During the 2nd and 3rd trimester of your pregnancy, as well as between 2 and 3 months after the birth of your child, we will ask you to:

- complete the diary over one 24-hour period (weekday or weekend);
- collect a single urine sample immediately after you have completed the diary;
- record the date and time you collected this urine sample in your diary, as well as the time since your last void.

	2nd, 3rd Trimester, and 2-3 Months After Birth Samples	
	Weekdays or Weekend	
	Tuesday	Wednesday
Diary		
Single urine sample		

Example: If you start your diary on Tuesday morning, you would collect your urine sample on Wednesday morning.

Note: Urine sample is collected at end of 24 hours of diary entries. Record time of urine collection AND record time of last void.

Diary Legend

Time:
Indicate the time of day.

Activity:
Please describe all of your activities during each interval and identify all the consumer products that you used. It is important to provide details.

Food or Drinks Consumed:
Please identify the foods and drinks you had during that time interval.

Heating:
If any food is heated in a can or in a plastic microwavable container, please note this in the description.

Packaging:
If you eat any packaged foods or drinks, please record the type of packaging. That is, place a check mark in the column if the food or drink was from any of the following types of containers:

a) cardboard (milk cartons, juice cartons, frozen dinners);
b) tin can;
c) glass jar;
d) plastic wrap (e.g., Saran Wrap™);
e) plastic container:
   - soft flexible plastic;
   - hard rigid plastic;
   - see-through;
   - recycle # and letters: For each plastic container, if available, please note the recycling number stamped onto the underside of the container and any letters (e.g., # 7 PC);
f) other: indicate any other type of container from which you consumed food or beverages.

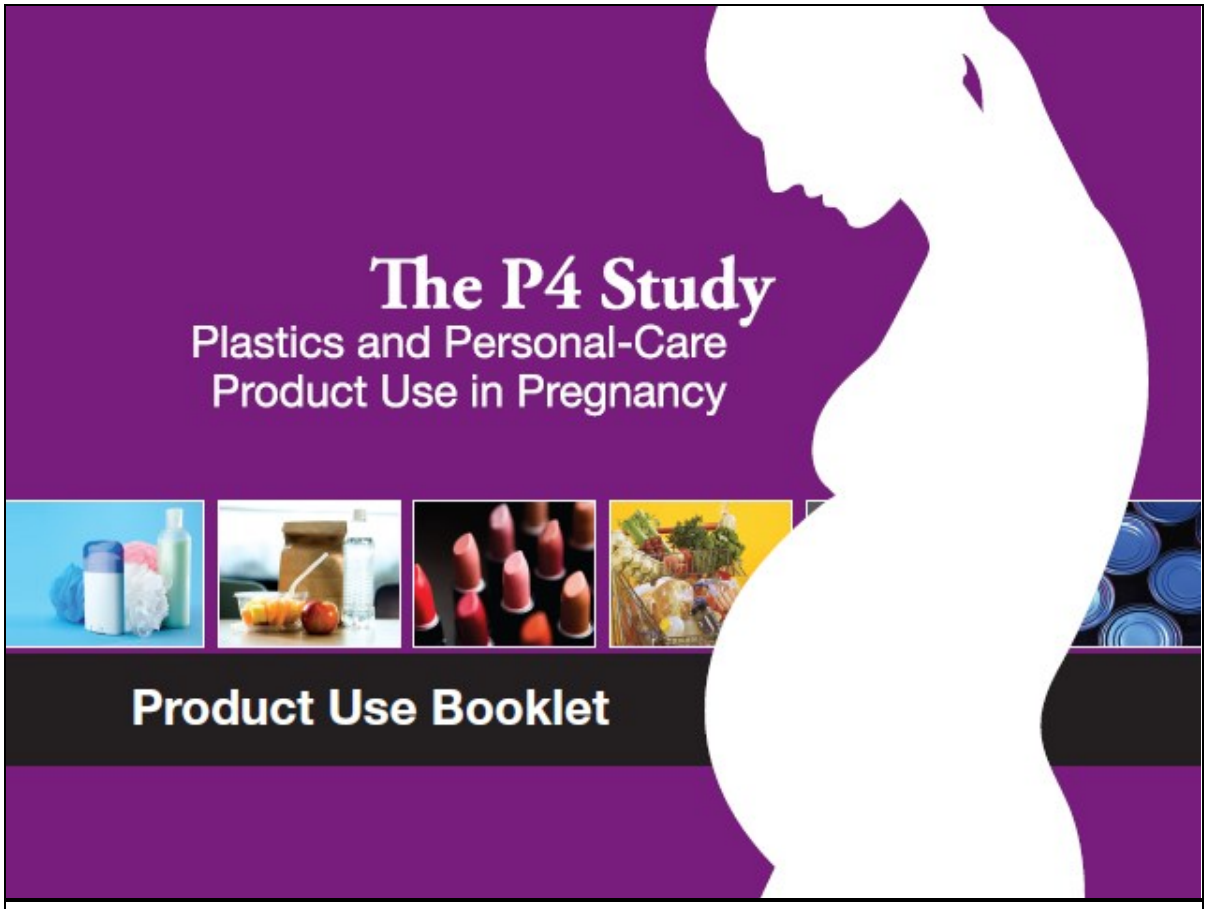
Date: _____

Time	Activities	Personal Care Products Used	Description of Food or Drink
6:00 am	Ate breakfast		Blueberries
	vitamins		Raspberries
			Cereal
			Milk
6:30 am	Brush teeth		Coffee
	Shower	Shampoo	
		Conditioner	
		Face Cream	
		Skin Cream	
		Deodorant	
		Lipstick	
		Mascara	
		Eye Shadow	
8:00 am	Drove to work		Coffee

Food or Drinks Consumed									
Type of Food or Drink Containers									
card-board or paper	tin can	glass jar	plastic wrap or bag	plastic				other	heated in container?
				soft flexible	hard rigid	see-through	recycle number		
				✓			6 PS		
				✓			3 V		
✓									
✓									
✓									
✓									

Date: _____

Time	Activities	Personal Care Products Used	Description of Food or Drink
6:00 pm	Made and ate supper		Chicken
			Rice
			Frozen peas
			Water (Brita jug)
			Milk
			Butter
			Ice Cream
7:30 pm	Bathed son	Baby shampoo	
		Baby lotion	
8:30 pm	Watched TV and snacked		Chips
9:00 pm	Vacuumed, washed kitchen floor		
10:00 pm	Loaded dishwasher		



Instructions for Product Use Booklet

You will complete the Food and Activity Diary for a total of 7 days throughout your pregnancy:

- 4 days in the first trimester (2 weekdays, and 2 week-end days)
- 1 day in the 2nd trimester
- 1 day in your 3rd trimester
- 1 day when your baby is 2-3 months old.

In this booklet we would like you to identify any consumer and personal-care products that you use with a daily activity as indicated in your diary. We would like you to record the product's:

- Manufacturer: the manufacturer is usually found on the bottom or the back of a product. Often it is accompanied with the manufacturer's mailing address.
- Complete brand name: this refers to the name of the product.
- Dates used: if used on the days that you completed your Food and Activity Diaries.

Please keep the Product Use Booklet throughout your pregnancy so you do not have to re-record your products with each new diary. Simply add any new products that you use as the study progresses.

Date enrolled in Study : __YYYY__ / __MM__ / __DD__
Study ID: _____

Date: ______________________

Product	Manufacturer	Complete Brand Name
Anti-perspirant	Manufacturer A	Anti-perspirant Name
Body Wash	Manufacturer B	Body Wash Name
Lipstick	Manufacturer C	Liptsick Name
Mascara	Manufacturer D	Mascara Name
Eye Shadow	Manufacturer E	Eye Shadow Name
Facial Cleanser	Manufacturer F	Facial Cleanser Name
Laundry Soap	Manufacturer G	Laundry Soap Name
Moisturizer	Manufacturer H	Moisturizer Name
Moisturizer	Manufacturer I	Moisturizer Name
Shampoo	Manufacturer J	Shampoo Name
Shampoo	Manufacturer K	Shampoo Name
Toothpaste	Manufacturer L	Toothpaste Name
Vitamins	Manufacturer M	Vitamin Name
Hand Soap	Manufacturer N	Hand Soap Name
Dishwasher Soap	Manufacturer O	Dishwasher Soap Name
Dishwasher Rinse Agent	Manufacturer P	Dishwasher Rinse Agent Name

* Please note that we have used fictitious names for the manufacturers and brands. It is important that participants supply the actual names.

Indicate the dates used below						
01/14	01/15	01/17	01/18	02/19	05/20	10/30
✓	✓	✓	✓	✓	✓	✓
✓	✓	✓	✓	✓	✓	✓
✓	✓	✓	✓	✓	✓	✓
✓	✓	✓	✓	✓	✓	✓
✓	✓	✓	✓	✓	✓	✓
✓	✓	✓	✓	✓	✓	✓
✓	✓	✓	✓	✓	✓	✓
✓	✓	✓	✓	✓	✓	✓
✓	✓	✓	✓	✓	✓	✓
✓	✓	✓	✓	✓	✓	✓
		✓	✓	✓	✓	✓
✓	✓	✓				
✓	✓	✓	✓	✓	✓	✓
✓	✓	✓	✓	✓	✓	✓
✓		✓		✓	✓	✓
✓		✓		✓	✓	✓

EXAMPLE

APPENDIX H – PERTINENT DATABASE COMPONENTS

- Questionnaires (T1a, T2, T3, T5)
- Chart review (T4)
- Journals (T1a, T1b, T2, T3, T5)
- Product Use Booklet
- Biospecimen Tracking Logs (date and time of sample, specific gravity)
- Specimen Results from INSPQ (Environmental Chemical results)
    - Maternal Urine (T1a, T1b, T2, T3, T5)
    - Infant Urine (T4, T5)*
    - Meconium (T4)*
    - Breastmilk and or Formula (T5)*
    - Air monitoring (T1a, T1b, T5)*

*This matrix is not pertinent for the purpose of the analysis of P4 triclosan data for this thesis; however, it was included in the overall P4 database.

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