Title of the Proposal:

"Project 500" CHILD Study

STUDY METRICS:

Planned number of subjects:	In total, the CHILD Study recruited 3,629 families and 3,350 children are currently being followed. **We plan to test several key hypotheses by analyzing 1 year outcomes in a subset of 500 children in whom we now have complete datasets to that age.**
How long will it take to write and submit protocol to IRB/IEC (in months)?	REB approvals for the full CHILD Study have been received at all four recruitment sites – Vancouver, Edmonton, Winnipeg, Toronto – and at the National Coordinating Centre in Hamilton. All are current. No additional ethical approval is required to undertake the analyses proposed.
Projected Start Date (<u>after</u> IRB/IEC and contracting are complete):	Project 500 will commence as soon as funding is obtained to perform the biological analyses.
Expected accrual rate (# of subject/month):	Recruitment of the full CHILD cohort was completed in 2012.
Projected enrollment timelines: *Please specify* - Date first subject enrolled (mm/yyyy): - Date Last subject enrolled (mm/yyyy): - Follow-up time (in months):	The cohort is fully enrolled. The 500 children selected for this project have all the required data and samples now available. Follow-up of all CHILD subjects is planned until infants are aged 5 years but this specific project is examining outcomes in the first year of life.
Target time of the first formal analysis (mm/yyyy):	June 2015
Target time of the final report (mm/yyyy):	June 2016
Time to generate draft manuscript or poster/abstract (in months):	3 months
Target Publication or Conference (include mm/yyyy):	Data will be presented at national (AllerGen, CSACI) and international (ATS, ERS) conferences 2015-16.

Purpose and rationale for the study:

This proposal relates to the testing of several specific hypotheses in a subset of 500 participants in the Canadian Healthy Infant Longitudinal Development (CHILD) Study. These 500 now have complete data from the time of recruitment (in pregnancy) to age 1 year. The primary purpose of this proposal is to identify risk factors for early allergic outcomes and biomarkers that may predict future disease. These 500 infants will provide critical preliminary data, not only related to early outcomes, but also to inform analytical plans for the full CHILD cohort.

Overview of the CHILD Study: The CHILD Study was established in 2008 in response to a CIHR RFA, and is now firmly established as a multicentre national Canadian cohort study. We have over 3,300 infants in follow-up, which will continue until at least age 5 years, and are now embarking on testing some of the many hypotheses built into the study, focusing on those related to early outcomes.

The primary objective of the CHILD Study is to better understand key relationships and interactions among the many genetic determinants and environmental exposures associated with the development of childhood asthma. Dramatic increases in atopy and asthma over just a few decades suggest new or changing environmental exposures, but the developmental origins of atopic disorders remain unresolved. We are investigating the root causes of asthma and allergy using a broad-based, longitudinal birth-cohort, which recognizes multiple environmental exposures (indoor and outdoor air pollutants, chemicals, infections, nutrition, psychosocial environment, drug intake), their timing (pre- and post-natal), and their interactions with genetics and epigenetics.

The overarching hypothesis of the CHILD Study is: ***The development of childhood asthma is dependent on the interactions of genetic predisposition and critical prenatal and early childhood environments including nutrition, physical and microbial environments, infections and social environment (socioeconomic status and stress).*** Within this overarching hypothesis, multiple sub-hypotheses will be tested within seven interrelated themes, including early life innate immune responses, pre-natal and post-natal nutrition and intra-partum care, lung function in early childhood and the impact of viral infections, early childhood socioeconomic status and life stressors, the impact of the physical environment, specific gene-environment interactions, and epigenetic mechanisms.

The objective of Project 500 is to carefully analyze a complete set of multiple clinical and environmental questionnaires and selected infant biological samples pertinent to early clinical outcomes (up to age 1 year). The **primary clinical outcomes are recurrent episodes of wheezing and development of atopy by age 1 year**. Atopy is defined by skin allergen responses. Secondary outcomes include atopic dermatitis or eczema, and reported food allergy. We propose detailed exploration of patterns of clinical illnesses and will correlate these with analysis of multiple biomarkers to determine their predictive power.

There is good evidence from longitudinal epidemiological studies and from clinical trials that allergy is both a common precursor to childhood asthma and also a predictor of persistence of childhood asthma into adulthood. The "atopic march" is a well recognized phenomenon in which children develop atopic dermatitis and/or food allergy in early childhood, and then go on to have recurrent wheezing and persistent atopic asthma in later childhood and adulthood. Our selection of specific hypotheses for examination in Project 500 reflects this phenomenon.

Four specific hypotheses will be tested in "Project 500":

1. **Maternal diet in pregnancy influences food sensitization in infancy; specifically avoidance of foods (e.g. peanuts) is associated with intolerance to that food.**

   Research on diet and interventions to prevent atopic disease have focused on foods with anti-inflammatory properties (e.g. n-3 fatty acids), antioxidants (vitamin E and zinc), and vitamin D. Recent

meta-analyses suggest beneficial effects for pre-natal vitamins A, D, and E, zinc, fruits and vegetables, and the Mediterranean diet. Nutrients may impact development of asthma through immune modulation or the child's intestinal microbiome. In spite of, or possibly because of, recommendations for early life avoidance of specific 'highly allergenic' foods including peanut in the 1990s from many national expert organizations (e.g. Canadian and British Pediatric Societies, American Academy of Pediatrics) there has been an explosion of food allergy, particularly to peanut in most high income countries (Miles and Buttriss, Nutrition Bulletin 2010). Intriguingly, studies have shown a very low prevalence of peanut allergy in Israel where peanut is commonly introduced in the first year of life compared with England where it is seldom introduced before the first birthday. This study accounted for a general heritable risk using children from a similar background (Ashkenazi Jewish) in both countries. (DuToit et al, JACI 2008). In the Danish National Birth Cohort maternal peanut (RR: 0.66; 95% CI: 0.44-0.98) and tree nut (RR: 0.83; 95% CI: 0.70-1.00) intake during pregnancy was associated with decreased risk of asthma in the child at 18 months of age. Intriguingly, there was no comment on the development of allergy in this cohort. The data we are collecting in the CHILD Study will allow us to examine the link between maternal intake of specific foods such as peanut and the outcome of allergy to that food in the infant.

2. **Low serum 25-hydroxyvitamin D values in infancy are associated with development of early childhood wheezing.**

   Most, but not all, studies have demonstrated a relationship of maternal vitamin D insufficiency with wheezing in pre-school children. A meta-analysis of four large cohort studies found that high maternal vitamin D intake during pregnancy was protective against wheeze in children. However, a recent publication from the ALSPAC cohort shows an association of increased wheeze with increased vitamin D levels. One issue may well be polymorphisms of the Vitamin D receptor in the mother (associated with vitamin D levels during pregnancy) and in their offspring during the few years of life. The data obtained in the CHILD Study will provide the opportunity to confirm or negate the associations between levels of vitamin D and wheezing syndromes in early childhood.

3. **Sensitization to cow's milk, egg or peanut, together or separately, at 1 year is a major risk factor for wheezing episodes in early infancy.**

   Sensitization to foods has been associated with an increased risk for asthma (Rhodes et al, J Allergy Clin Immunol. 2001;108:720-5 and has recently been included in a modified Asthma Predictive Index (mAPI) (NEJM 2006;354:1985-97). The mAPI and an m2API have been validated in a small cohort as good predictors for persistent asthma at school age (Chang T et al, J Allergy Clin Immunol: In Practice 2013; 1:152-156). As yet unpublished data from a Canadian cohort show a substantial increased risk (OR 8-10) of persistent asthma in children with sensitization to a food in the first year of life (verbal communication, Becker A). We will be able, using CHILD Study data, to examine these early life sensitization patterns in relation to the occurrence of wheezing episodes, and not only determine immediate relationships but also follow the children for several years to validate (or not) the Asthma Predictive Index.

4. **Exposures to oxidizing agents in the prenatal period and during the first 3 months of life influence the development of atopy and wheeze at 1 year.**

   Infants raised in Canada spend a large majority of time indoors at home. Multiple indoor air exposures have been associated with wheeze and asthma, environmental tobacco smoke (ETS) being the most widely recognized. Oxides of nitrogen, associated with gas cooking and heating, are linked with worsening asthma. Innate immune inflammatory response can be activated by common indoor exposures, including trichloramines, aldehydes (e.g., formaldehyde) and other volatile organic compounds (VOCs) in home furnishings and cleaning products.

Recent studies suggest that early-life exposure to ambient air pollution may also contribute to the development of asthma, with traffic-related air pollution (TRAP) likely playing an important role. TRAP represents a complex mixture of pollutants (particulate matter, nitrogen oxides, carbon monoxide, organic compounds) in concentrations that are variable over short distances, but that also build-up over parts of the city contributing to a variable urban background. Respiratory health risks are generally highest among those living close to busy roads with significant truck traffic, but concentration variations across urban areas have also been associated with incident asthma. The effects of TRAP on airways disease likely also depend on poorly understood interactions with a range of endogenous or exogenous factors such as genetics, co-exposures such as allergens and environmental tobacco smoke, and psychosocial stress. The CHILD Study provides an opportunity to study TRAP in four major cities with highly variable and well-characterized concentrations, and to examine relationships between TRAP, indoor air contaminants, and development of wheeze and atopy in early childhood with the intent of following these children through childhood to determine asthma outcomes.

Description:

Primary Objective(s) and Endpoint(s), *if different from objective(s)*	**The primary objective of the full CHILD Study** is to better understand key relationships and interactions among the many genetic determinants and environmental exposures associated with the development of childhood asthma, and use this understanding to prevent it and improve its treatment. Identification of causal pathways will lead to development and implementation of preventative and therapeutic strategies to reduce morbidity and even mortality of childhood asthma. **The primary objective of Project 500** is to analyze multiple clinical and environmental questionnaires and selected infant biological samples to determine predictors of early clinical outcomes related to allergy and asthma, utilizing a **subset of children who now have complete data to age 1 year**.
Secondary Objective(s) and Endpoint(s), *if different from objective(s)*	**The CHILD Study** has been developed as a national Canadian resource, currently supporting and linking many research projects within the AllerGen Network of Centres of Excellence, and supporting several CIHR-funded projects including investigation of the role of the human microbiome. The cohort, as individuals and through the availability of the detailed database and collection of biological samples, will be a platform for not only allergy and asthma related research, but potentially for many other investigations of the childhood origins of chronic adult diseases, including obesity, cardiovascular and metabolic disease. In addition, the availability of clinical data on some 6000 parents of the index children will provide fertile material for multiple investigations in adults, as well as potentially providing resources for future clinical trials. Completion of Project 500 will provide sound data on which to base further investigations in the full cohort. We propose to explore the relationships between early childhood clinical and biomarker outcomes and development of overt chronic diseases – asthma and the many other manifestations of allergic disease, and cardiovascular and metabolic disorders such as obesity and diabetes. We will examine relationships between maternal nutrition and infant/childhood health, infant nutrition and childhood health, and interactions in multiple fields between genetics and the environment including the role of epigenetics.

Study Design	**CHILD Study Methods:** Pregnant women from the general population were recruited in the second and third trimester of pregnancy in multiple urban and rural centres; they reflect ethnically, environmentally, socioeconomically and culturally diverse families with over 25% non-Caucasian parents. Children currently range from those recently born up to 5 years old. Children are assessed at delivery, at a 3-month home visit, and at 1, 3, and 5 years of age. Repeated and diverse environmental and health questionnaires combined with objective measurements (allergy skin prick tests, lung function) detailed home inspection, and a rigorously annotated bank of biological samples from parents (venous blood, breast milk), index child (cord and venous blood, stool, urine, nasal secretions) and home (dust) provide a robust, comprehensive database for testing of multiple interrelated hypotheses. **Specific methods pertinent to the current analyses:** Data entry related to events prior to the 1 year assessment have been completed as a priority for these 500 children, and data entry for all 1 year questionnaires and samples given priority. Questionnaire data will then be accessed from HealthDiary, cleaned and assembled by an experienced Data Manager. 500 dust samples and 1500 blood samples (mother, cord blood, child at 1 year) will be identified and retrieved for analyses. Dust analyses will be undertaken at the laboratory of Dr James Scott, University of Toronto, and blood analyses at the Clinical Trials and Clinical Research Laboratory, Hamilton. Teams of CHILD investigators will analyze the resulting data in collaboration with local statisticians and with our Senior Statistician, Dr Wendy Lou, Canada Research Chair in Statistical Methods for Health Care, University of Toronto. **[1] Maternal diet in pregnancy influences food sensitization in infancy, specifically avoidance of foods (e.g. peanuts) is associated with intolerance to that food.** Pre- and post-natal nutrition has been collected using maternal and infant/child questionnaires including exclusive and non-exclusive breast-feeding, age of first supplementation and age of introduction of solid foods. A Food Frequency Questionnaire (FFQ) developed by nutritional epidemiologists at the Fred Hutchinson Cancer Research Center, based on questionnaires used in US studies was modified to reflect Canadian ethnic food choices. Further, the database developed by the University of Minnesota Nutrition Data Systems for Research for data entry and nutrient analysis was "Canadianized" for use in CHILD. This self-administered FFQ asks pregnant mothers to report the frequency of consumption and portion size of approximately 175 line items during their pregnancy, and was completed by the mother at the time of enrollment. At age 3 and 6 months and 1 year, child diet was recorded, including exposure to cow's milk, eggs and nuts. **[2] Low serum 25-hydroxyvitamin D values in infancy are associated with development of early childhood wheezing.** Serum is obtained from children at age 1 year; vitamin D will be measured by standard assay methods at the research laboratories at the Hospital for Sick Children. Recurrent wheezing will be identified from questionnaires completed by mother when the child is 3, 6 and 12 months of age.

	[3] Sensitization to cow's milk, egg or peanut, together or separately, at 1 year is a major risk factor for wheezing episodes in early infancy. Epicutaneous testing at 1 year of age is performed to common food allergens including cow's milk, egg and peanut. A positive test (≥2 mm wheal response greater than the negative control) will define sensitization regardless of the presence of a positive history of an allergic reaction (i.e. history negative skin test positive). A definition of food allergy requires the presence of a positive test for allergen specific IgE and a positive history of a clinical allergic reaction or, in the absence of known exposure to that food, a positive skin test and an ex vivo measurement of allergen-specific IgE at a level defined to represent the 95th percentile or higher for a positive challenge to that food. **[4] Exposures to oxidizing agents in the prenatal period and during the first 3 months of life influence the development of atopy and wheeze at 1 year.** **Data sources:** A comprehensive baseline questionnaire (during pregnancy) and updates at 3 months, 1, 3, and 5 years of age record residential address, housing structure, function, condition, maintenance and cleaning habits, presence and use of attached garage, renovations, source and extent of dampness, mold growth, new furnishings, appliance and household cleaner emissions, presence and type of air conditioning and cleaning, and conditions of use. Questions related to the child's and families' time activity include time spent in different rooms in the house and indoors vs. outdoors, time in transit, mode of transport, frequency/duration of visits to daycare, indoor pools and exposure to smoke. Shorter questionnaires at 6 and 18 months, 2, 2.5, and 4 years of age focused on child time-activity and major changes including renovations. When the infant was 3-4 months old, research staff trained by Canada Mortgage and Housing Corporation and the CHILD Environmental Working Group visited the home. This enabled measurement of geographical coordinates (GPS) for assigning air pollution and traffic-related air pollution (TRAP) exposure, evaluation of the structure, function and exposure sources within the home with emphasis on cleanliness and cleanability, furnishings, ventilation (air conditioning, heating, cooling), potential for moisture build-up, microbial and chemical contaminant burdens, basement conditions and potential for air exchange with the outside and with attached garages. The breadth of exposures characterized will enable development of novel exposure indices for integrating multiple oxidative and/or inflammatory exposures to better capture total risk posed by the physical environment. **Indoor Exposures:** Questionnaires included animal exposures (dogs, cats, other pets) at multiple time points. Dampness (water leaks) and mold were also assessed by repeated questionnaires, and visual inspection of the home. Home (most used room) and child's bedroom dust was systematically sampled with a specially designed vacuum system, weighed, sieved and stored at -80C. A subset of the fine dust has been analyzed for endotoxin and β-glucans to support questionnaire validation and application in regards to dampness and mold. A range of organic chemicals are also being quantified (e.g., phthalates, PAHs) at Environment Canada laboratories. We propose to analyze dust for dog, cat, and house dust mite allergens; all children are skin-tested for these and other inhalant allergens at 1, 3 and 5 years of age. Urine collected at 3

	months and 1 year in a subset of the cohort is currently being analyzed to assess exposure to environmental tobacco smoke and phthalate plasticizers. **Outdoor exposures:** We have characterized both large scale patterns and the small-scale spatial gradients in TRAP and other combustion-related pollutants within each city. With home GPS coordinates, and addresses of locations the children frequently visit (e.g., daycare) we can assign a time-weighted air pollutant exposure. Land use regression (LUR) models have been developed for each city to predict exposure to TRAP. The National Air Pollutant Surveillance Program (NAPS) monitoring data and Environment Canada air quality model objective analyses are available to capture the larger scale patterns. LUR models are consistent across seasons. Time activity data, obtained every 3 months in year 1, provides weighting to these two levels of outdoor exposures.
Statistical justification for the study design, sample size calculation, and statistical analysis plan	**For Project 500, we have selected 500 children in whom we now have complete data to age 1 year.** These will comprise approximately 125 children from each of the recruitment sites to minimize geographic bias. At age 1 year, the dominant clinical outcomes of relevance are wheezing (as defined as a positive response to ever wheeze on 3, 6 or 12 month questionnaires), atopic dermatitis and reported food allergy, and objective evidence of sensitization to food and inhalant allergens assessed by skin testing (≥2mm wheal response to epicutaneous testing). We currently have little information on biological data at 1 year, as it is rare for normal children to have blood taken at this age. It is even less common to have a population of healthy normal children providing blood, urine, stool, nasal secretions, and skin allergy tests, to examine correlates of biomarkers with these outcomes. Hence power calculations for this sample of 500 are difficult, but the planned analyses will provide preliminary data which will allow calculations of appropriate sample sizes for future analyses in the full cohort. We anticipate approximately 50 of these 500 children will have experienced wheezing in the first year of life, and a similar number will have developed atopic dermatitis. **For the full CHILD Study**, the primary outcome is physician-confirmed asthma at age 5 years, with multiple pre-specified intermediate and secondary outcomes. Recruitment and follow-up of over 3,000 families from the general population will allow well-powered integrated analyses involving derivation of new multi-exposure indices; examining the effect of respiratory infections on growth of infant pulmonary function; identification of innate and adaptive immune phenotypes in relation to environmental exposures; linkage of epigenetic profiles to maternal stress, socioeconomic status, environmental exposures and viral infections; and determining impact of nutritional factors including maternal diet, breast-feeding, infant diet, transition to table foods and the role of probiotics and nutraceuticals on immune function through the infant microbiome. The comprehensive longitudinal assessments within CHILD allow sophisticated statistical modeling, including longitudinal latent class analysis, structural equation modeling and factor analysis to assess specific pathways relating genetics, SES, immunity, environment and nutrition to the development of allergy and asthma. **The statistical analyses for Project 500** will include a number of statistical approaches, including logistic regression (Agresti, 2012), generalized linear models, and generalized linear mixed models (McCulloch et al., 2008). Both

	univariate and multivariate analyses will be employed, and the effects of potential confounders will be investigated through multivariable analyses. All exposure and outcome variables will be first examined, prior to statistical modeling. For continuous measures (e.g. vitamin D intake during pregnancy in Hypothesis 1, serum 25-hydroxyvitamin D in infancy in Hypothesis 2, indoor concentrations of endotoxin and β-glucans in Hypothesis 4), the distributions (including mean, median, range, variance, etc) will be described and then used for determining the appropriateness of possible modifications to measures (e.g., log transformation for non-normality, or categorizing values using cut-offs based on both statistical and clinical/biological reasons); for categorical measures (e.g., development of wheezing at 1 year in Hypothesis 2, positive results for peanut sensitization in skin tests in Hypothesis 3, and development of atopy at 1 year in Hypothesis 4), frequencies and percentiles will be summarized and used to determine choices of statistical methods (e.g., exact methods for sparse data, if applicable). Specifically, for **Hypothesis 1,** the association between intolerance to certain foods (e.g. peanut) and maternal diet (e.g. exposure to nuts) will be examined first using bivariable logistic regression analysis; here the outcome (binary) variable is intolerance (e.g. to peanuts, yes/no), and the explanatory variable is diet (e.g. avoidance of peanuts, n-3 fatty acid intake), which can be either a binary (yes/no), a categorical (low/medium/high) or a continuous variable. When considering the effects of covariates, such as gender and maternal atopy, on the association being investigated, multivariable logistic regression analysis will be employed, and potential confounders such as ethnicity will be accounted for in the regression model. Similar approaches will be employed in the analyses of the other hypotheses. For **Hypothesis 2**, the outcome variable, development of early childhood wheezing (yes/no), will be determined at 1 year, and the measure of serum 25-hydroxyvitamin D status [25(OH)D] will be examined for its distribution and, if necessary, categorized as an ordinal variable (e.g. low/medium/high based on statistical and clinical/biological thresholds). Results based on ordinal categorization will likely provide an intuitive interpretation of the association (e.g. quantifying an n-fold increase in risk for developing childhood wheezing when comparing high vs. low 25(OH)D). As a primary exposure variable of interest, the distributions of 25(OH)D values will be compared between two groups, infants with vs. without wheezing, using nonparametric methods (e.g. Wilcoxon rank-sum test) and the t-test (if normally distributed); if the distribution exhibits a categorical trend, the chi-square test will be applied to evaluate the relationship with the outcome (childhood wheezing). For **Hypothesis 3**, the association between the outcome variable, wheezing episodes in early infancy (any wheeze to 1 year), and sensitization to foods (positive test result) will be examined using regression models for categorical data. As described in the previous section “Study Design”, sensitization will be defined as a binary variable (positive/negative), and test results for single and multiple foods (e.g. sensitivity to egg and peanut) will be considered in the analyses, which will employ multiple indicators (so-called “dummy variables” for each of the foods tested). To investigate whether there are additive effects that combinations of sensitizations to different foods have on the frequency of

	wheezing episodes, the generalized linear models and the generalized linear mixed models will be utilized. When considering the exposures to oxidizing agents under **Hypothesis 4**, the primary outcome variable is the development of atopy and wheeze at 1 year (binary, yes/no). The relationships between the outcome and the various outdoor and indoor exposures (e.g., home dust mite allergens, continuous variable; pet ownership, categorical variable) described in the Study Design will be investigated using multivariable logistic regression analyses. The effects of other variables, such as genetics and psychosocial stress, will be examined in the regression model as covariates. For all four specific hypotheses, the statistical evaluations will include univariate (e.g. distribution of 25(OH)D), bivariate (e.g., association between sensitization to peanut and childhood wheezing), and multivariate (e.g., relationship between the development of early childhood wheezing and low serum 25(OH)D value while controlling for birth weight) analyses. Variable selection, including possible interactions (effect modifications) between variables, for the multivariable regression models will be adjudicated based on both statistical and clinical significance, and the final model will be determined using the Akaike Information Criterion and the Hosmer and Lemeshow goodness-of-fit test (Hosmer and Lemeshow, 2000). All of the approaches mentioned above can be readily implemented using SAS (v9.3) and/or R (v2.15).
Study Population , and key Inclusion /Exclusion criteria	**The 500 children in Project 500 will be representative of our full cohort.** Study members have been recruited from a general population (i.e., not high-risk for asthma and allergy as are many cohorts) from recruitment bases in Vancouver, Edmonton, Winnipeg (including a rural site in Manitoba) and Toronto. **Inclusion Criteria** 1. Pregnant women aged 18 years and older (19 in Vancouver) 2. Residence in reasonable proximity to the delivery hospital 3. Able to read, write, and speak English 4. Willing to provide informed consent 5. Willing to consent to cord blood collection for the study 6. Planning to give birth at a designated recruitment centre participating hospital 7. Infants born at or after 35 weeks 8. Able to provide address and telephone number and names and telephone numbers of two alternate contact individuals **Exclusion Criteria** 1. Children with major congenital abnormalities or respiratory distress syndrome (RDS) 2. Expectation of moving away from a recruitment area within year 1 3. Children of multiple births 4. Children resulting from *in vitro* fertilization 5. Children who will not spend at least 80% of nights in the index home

	The families recruited do not include Children in Care, as the essential component of the study is determining gene-by-environment effects on development of asthma and allergy. This includes the in utero and early home environment, and parental genotyping and phenotyping, requiring known birth parents and as far as possible a relatively constant environment for optimum assessment.
Treatment information, *if applicable*	The study is observational, with no planned treatments or interventions.
Study procedures in place to ensure proper conduct of the study according the principles of ICH/GCP	All procedures and components of the study have been standardized across centres, with agreed SOPs for all major components. The Research Project Manager makes regular site visits to ensure compliance with SOPs, monitor completion of questionnaires and all related data including sample handling and labeling. The National Coordinating Centre (NCC) checks all REB requirements are met and signed approval given. Data are entered largely at the NCC, with procedures for ensuring confidentiality and anonymity of data.
Study Procedures and Assessments	Quality control procedures are in place for verifying all questionnaire data (checking by local research assistants, checking during data entry, queries to sites for missing or incomplete data) and biological samples (all divided into multiple aliquots to avoid freeze/thaw issues, each aliquot individually identified and tracked). Many questionnaires are now answered electronically via a secure website, but remain subject to rigorous checking.
Details of Clinical Safety Reporting Processes *(when applicable provide specific risk management interventions to safeguard subjects)*	Not applicable, except that if there should be observation of an obvious danger to the child, or instances of child abuse, or other reportable issues noted in the family, such would be drawn to the attention of the appropriate authorities. To date there have been no such situations identified.

Study Location:

Specify all location(s) where the study will be conducted, incl. Country, name and full address of the institution(s). *Copy and paste the below fields as needed in order to provide contact details for all participating sites.*

Investigator's name	Malcolm R Sears on behalf of the CHILD Study
Name of Institution	McMaster University / AllerGen NCE
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Investigator's name	Padmaja Subbarao (Toronto site leader)
Name of Institution	Hospital for Sick Children

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Investigator's Name	**Investigator's Institution**
Andrew Sandford	University of British Columbia, Vancouver, BC
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Michael Brauer	University of British Columbia, Vancouver, BC
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Tobias Kollmann	University of British Columbia, Vancouver, BC
Timothy Takaro	Simon Fraser University, Vancouver, BC
Anita Kozyrskyj	University of Alberta, Edmonton, AB

Dean Befus	University of Alberta, Edmonton, AB
Kent HayGlass	University of Manitoba, Winnipeg, MB
Felix Ratjen	The Hospital for Sick Children, Toronto, ON
Sanja Stanojevic	The Hospital for Sick Children, Toronto, ON
Theo Moraes	The Hospital for Sick Children, Toronto, ON
Jeffrey Brook	Environment Canada, Toronto, ON
James Scott	University of Toronto, Toronto, ON
Richard Hegele	University of Toronto, Toronto, ON
Wendy Lou	University of Toronto, Toronto, ON
Susan Elliott	University of Waterloo, Waterloo, ON
Judah Denburg	McMaster University, Hamilton ON
Joseph Macri	McMaster University, Hamilton ON
Catherine Laprise	Université du Québec à Chicoutimi, Chicoutimi, PQ

Estimated Study Budget:

The primary costs of Project 500 are related to data and sample analyses. We are collaborating with the Canadian Laboratory Initiative in Pediatric Reference Intervals (CALIPER) project to obtain best costs for laboratory analyses on our small volume samples.

This application is for $200,000 to be applied as detailed below.

Is monetary funding requested?

YES:_____ *If yes, give details* (per subject costs, cost of study medication, cost of other supplies, other):

This application is for a grant of $200,000 in fiscal year 2012-13 to fund the following components of Project 500 of the CHILD study related to analysis of biological samples and clinical data to facilitate determination of biomarkers of disease	
Data entry of clinical and biological data from 500 children aged 1 year, 3 hours per child for all forms and sample data, data entry clerk rate average $18.00 per hour plus 14% in lieu of benefits (i.e. $20.52): 500 x 3 x $20.52	**$30,780**
Data manager for 6 months – annual salary $95,000 plus 24% benefits x 0.5	**$58,900**
Retrieval of 1500 blood samples from liquid nitrogen storage @ $2.50 each	**$ 3,750**
Vitamin D, 1500 samples @ $22 per sample	**$33,000**
Specific IgE (egg, peanut) in 500 blood samples (1 year child) @ $25 per sample	**$25,000**
Serum ferritin, 1000 samples (mother, child at 1 year) @ 13 per sample	**$13,000**
Serum folate, 500 samples (mother) @ $23 per sample	**$11,500**
Total serum IgE, 1000 samples (cord blood and 1 year) @$15 per sample	**$15,000**
Serum IgA (cord blood), 500 samples @ $15	**$ 7,500**
Supplies and shipping	**$ 1,570**
Total request: $200,000	

Is non-monetary funding requested?

NO:____ *If yes, give details:*

Not applicable

Has funding been received, requested or is intended to seek from any other source?

YES:_____ *If yes, give details:*

The CHILD Study was initiated with parallel grants from CIHR and AllerGen NCE, each committing $6,000,000 over 6 years. Further funding has been obtained through grants from Health Canada related to sample and data analysis to inform the Chemical Management Plan. Funding from AllerGen has been renewed at $2,000,000 per year from April 2012 for 2 years (2012-2014). Grant applications to CIHR and other agencies will be pursued for continued follow-up of the cohort and for specific data and sample analyses to address the multiple research questions.

Date: 15 April, 2013