Placental Uptake and Transfer of Environmental Chemicals Relating to Allergy in Childhood Years

PLUTOCRACY

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Abstract

The primary objectives of the study were to investigate whether neonatal (cord blood) cytokines and maternal cytokines at delivery were predictive biomarkers of allergic outcomes in children at 18months of age, and also to evaluate if allergy outcome in the child is affected by the pollutants. The secondary objective was to assess the effect of pollutants on immune status of the mother, placenta cytokines, cord blood cytokines and child cytokines.

Multiple regression models, Multiple logistic regression models and Multivariate logistic regression models were fitted to the data. Further, Receiver Operating Characteristic curves were used to determine the predictive ability of cord blood cytokines, maternal cytokines and pollutants for allergic outcomes.

A model cumulating allergies disclosed that, increased levels of sum of Organochlorine insecticides in breast milk (BM-sum3), Lead in placenta (Pbplacenta) and il4hacord cytokines increase the probability of manifesting atleast one of the allergies. Higher levels of the sum of Organochlorine insecticides in placenta (PLAT-sum3) and Lead in maternal blood serum (Pbblood) reduce chances of manifesting atleast one allergy in child. The probability of manifesting atleast one allergy is predicted by variables region, il4hacord, BM-sum3, Pbplacenta, PLAT-sum3 and Pbblood.

Multivariate models revealed that, higher levels of il4phacord, il5phacord, il10phacord and INFgmaternal increase the probability of manifesting all four allergies. In the presence of Phadinhalant, children in Slovakia have a lower probability of having all allergies than children in Belgium. Elevated levels of INFgphamaternal in the maternal blood serum increase the probability of developing all four allergies in the absence of Phadinhalant. Increased concentrations of Cadmium in placenta, sum of Organochlorine insecticides in maternal blood and sum of Organochlorine insecticides in breast milk increase the probability of developing all four allergies. Higher levels of Lead in maternal blood serum, sum of Chlorinated benzenes in maternal blood serum, sum of PCBs in breast milk, sum of PCBs in maternal blood serum and sum of PCBs in placenta reduce the probability of developing all four allergies.

Multivariate models confirmed that cytokines il4phacord, il5phacord and il10phacord predict Atopic eczema. Also, INFgphamaternal cytokines and country predict food allergies and Atopic eczema. Lead in maternal blood serum, sum of PCBs in placenta, sum of PCBs in breast milk, sum of Organochlorine insecticides in breast milk, sum of PCBs in maternal blood serum, Cadmium in placenta and sum of Organochlorine insecticides in maternal blood serum predict Atopic eczema in child.

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1 Introduction

1.1 Background

Placental contamination with xenobiotics (chemical compounds that are foreign to bodies of living organisms) represents a bioindicator of the environmental exposure of the mother, and may also have an impact on intrauterine fetal development and postnatal health status of children. Many foreign substances can reach the developing fetus through placental transfer. Accumulation of xenobiotics in the placenta may result in deterioration of immunological profile of the placenta which affects the health status of a child. In this study, xenobiotics assessed for contamination of the placenta include Lead, Cadmium, Chlorinated benzenes, Polychlorinated biphenyls (PCBs) and Organochlorine insecticides. The concentration of these xenobiotics in the placenta will then be investigated for allergy outcomes.

Allergy development is the result of environmental exposure to risk factors and genetic back ground of an individual. In childhood years, allergy refers to the genetic potential to manifest allergic diseases such as Atopic dermatitis. However, given the increased prevalence of allergic diseases in the recent decades it is unlikely that genetic factors alone are responsible for increasing trends. The interplay of the genetics with various environmental exposures may manifest in the form of allergic phenotypes (Von., (2004)). Thus the role of environmental exposures in the manifestation allergy outcomes needs to be examined to check the increasing trends of allergic diseases, which is the purpose of this report.

Among the environmental exposures is pollution which has been associated with progressive industrialization and agricultural by-products (Noakes *et al*., (2005)). Increasing levels of industrial and agricultural by-products such as Polychlorinated biphenyls and organochlorines contaminate homes, food, clothing and water sources. Exposure to these by-products at high doses in humans suppresses immune responses (Daniel et al., (2001)) which favours allergic diseases. In this study participants from two agricultural regions (Giurgiu and Stara Lubovna) and three industrialized regions (Mol, Bucharest and Bratislava) were included to assess whether allergy outcomes in children are associated with industrial and agricultural by-products.

Of concern in the current study, various organic compounds have been measured in maternal blood serum, placenta and breast milk. Moreover, one Eastern Europe group (Reichrttova *et al.*, (1999)) noted that higher levels of concentration of organic compounds, Polychlorinated biphenyls in particular, in cord blood were associated with higher levels of cord blood Immunoglobulin E (IgE) antibodies. These observations high light organic compounds as possible candidate factors of the allergy epidemic.

In the midst of genetic factors of an individual, concern was placed on immunity status of the mother, Th1 (INF-gamma, il2 and il12) and Th2 (il4, il5 and il10) cytokines as potential factors for allergy development. Indeed, elevated status of Th2 is associated with allergy outcomes in children at 24months of age (Duramand *et al*., (2006)). Further more, a study by Allam *et al*., (2005) demonstrated an association between elevated cord Immunoglobulin E (IgE) and development of allergic diseases. Also a study among neonates in the Slovak Republic, Reichrtova *et al*., (2002) reported a positive association between cord IgE and allergy outcomes.

Several interventions have been made to prevent allergic outcomes. Nevertheless, allergy outcomes are still a problem despite the efforts made. Studies by Simpson *et al*., (2005) revealed that the use of allergen proof encasings (mattress, pillow and duvet) as a single intervention in adults with asthma did not show any difference between the control and active groups over a 12-month period. In children, the use of encasings was associated with a reduction in asthma medication usage. A multifaceted intervention study, with the intervention tailored to the child's sensitization status and home environment, resulted in significant reductions in emergency room visits and symptoms in the active group. There is evidence from these studies that interventions in children (either single or multifaceted) are associated with a meaningful and sustained improvement in asthma control. However, for adults, allergen proof encasings as a single intervention cannot be recommended. Thus, there is need for interventions that can be meaningful and sustainable in all groups of the population. In the present study, focus is put on environmental exposures and genetic factors of mothers and children so as to come up with interventions of allergic outcomes associated with the environment and the genetics of an individual.

Although some researches claim sustainability of allergen interventions for specified groups, clinical trials of allergen avoidance tend to be small and the findings inconsistent. As a result, larger studies are recommended to make a review of the existing literature timely **(**Syed *et al*., (2006)**).** In the current study, we have 500 mothers and 500 children from three countries: Slovakia, Romania and Belgium. Five regions were selected from these countries: Slovakia (Bratislava & Stara Lubovna), Romania (Bucharest & Giurgiu) and Belgium (Mol). In addition the study comprises of 157 variables with Atopiceczema, Other dermatitis (Otherdet), Specific IgE for inhalant allergies in child blood (PhadCHinhalant) and Specific IgE for food allergies in child blood (fx5Chfood) as allergic outcomes in children at 18 months of age. Emphasis has been placed on cord blood cytokines, maternal cytokines, placenta cytokines, child cytokines and pollutants as possible risk factors of allergic outcomes in children. Further, TotalIgE, Atopic-status and Allergic history of mother were considered as allergic outcomes among mothers (genetic background of a child).

1.2 Objectives of the study

Since the placenta serves as an interface between the fetus and environmental exposures, this study seeks to investigate associations between environmental chemicals transferred through placenta and allergy outcomes in childhood years. The study was designed to investigate whether neonatal (cord blood) cytokines are predictive biomarkers of allergic outcomes in children at 18months of age, examine if maternal cytokines at delivery are predictive biomarkers of allergic outcomes in children at 18months of age, and also to evaluate if disease outcome in the child is affected by the pollutants. Our secondary aim was to assess the effect of pollutants on immune status of the mother, placenta cytokines, cord blood cytokines and child cytokines.

1.3 Organization of the report

The study design and materials used are introduced in Section 2. In Section 3 the data are described and the Statistical methodology is briefly explained in Section 4. Results from the methodology applied in Section 4 are presented in Section 5 and Discussion and, Conclusion and Recommendation in Sections 6 and 7 respectively.

2 Study design and materials

2.1 Study design

A prospective cohort of mothers/newborn babies was established in three European countries – Belgium, Romania and Slovakia. The three were chosen because they are the home countries of the different partners. Areas with different pollution pressure were targeted and exact locations were selected based on collaboration with local hospitals. Hospitals were selected for recruitment based on the birth rates and willingness of medical workers to collaborate in the project. Five regions were selected for the study according to environmental characteristics of Urban and Agricultural. One region was selected from Belgium, two from Romania and two from Slovakia as listed in Table 1 below.

Table 1: Regions selected and their environmental characteristics

Country	Region	Environmental Characteristic
Belgium	Mol	Urban
Romania	Bucharest	Urban
	Giurgiu	Agricultural
Slovakia	Bratislava	Urban
	Stara Lubovna	Agricultural

Belgium
Mol region with over 32,500 inhabitants is situated in the Northern Flemish part of Belgium and represents an urban region. One maternity hospital in Mol was selected for recruitment of pregnant women. Industries characterized by a nuclear centre and non-ferrous smelters are the main sources of pollution in Mol.

Romania
Bucharest, the capital of Romania represents the urban / industrial region and produces over 25% of Romania's industrial production. Bucharest has over 2 million inhabitants. Giurgiu with approximately 75,000 inhabitants represents an agricultural region. The main sources of pollution in Romania include industry, domestic sanitation, agriculture, traffic and combustion of plants for central heating and individual heating systems. One maternity hospital was selected from Bucharest and another from Giurgiu.

Slovakia
Bratislava with over 500,000 inhabitants represents an urban region. The main sources of pollution in Bratislava are heavy traffic, chemical industries and power generation. Due to low birth rate and relatively high percentage of pathological pregnancies in this region, three hospitals were considered for recruitment. Stara Lubovna is a rural region with approximately 15,000 inhabitants .The main source of pollution in this region is traffic resulting from a close border point to Poland, increased pollution during winter from local heating. Only one hospital in this region was involved in recruitment of participants. Altogether four hospitals were selected for recruitment in Slovakia.

2.2 Enrolment criteria

Mothers were approached by a trained nurse, informed about the study and signed an Informed Consent.

A total of 788 pregnant women were recruited before delivery from maternity hospitals according to the following criteria. The PLUTOCRACY project requested recruitment of healthy babies that had normal gestations and normal births. Mothers were not to move outside the study region after birth. Babies were to be between 37 – 42 weeks at the time of birth and had no prenatal pathology that would result in referring the child to another hospital during 48 hours after delivery. More specific guidelines for exclusion from the study included the following categories.

Mother
Mothers with chronic autoimmune diseases such as multiple sclerosis, diabetes, rheumatoid arthritis and those who took medication during the last three weeks of pregnancy and/or during delivery were excluded from the study. Medications included Tocolytics, Corticosteroids (orally, intravenously and intramuscular), General anesthesia and Insulin (during any time of pregnancy).

Child
Babies less than 37 weeks and weighed less than 2500 grams at the time of delivery were excluded. Further, babies with severe postnatal complications (pulmonary problems, infections, asphyxia, neurological, jaundice requiring exchange transfusion, e.t.c.) and congenital malformations (heart, bowel e.t.c.) were not recruited in the study.

Mother and Child
Mothers or babies were excluded if either or both of them showed any signs of infections in prenatal and postnatal period. Such infections included Pyelonephritis (Kidney infection), Gonorrhea, Syphilis/HIV, Chlamydia, Trichomonas, Herpes, Sepsis, Pneumonia and Meningitis.

Other exclusion in prenatal and postnatal period included Gestational diabetes, Chronic hypertension (high blood pressure before 20 weeks' gestation), Pregnancy- induced hypertension/preeclampsia (high blood pressure after 20 weeks' gestation), Oligohydramnios (too little amniotic fluid) and Polyhydramnios (too much amniotic fluid).

Out of 788 recruited, only 500 mothers turned up for postnatal examination when child was 18months of age. These were the participants who completed the study and for this reason considered for Statistical analysis. Distribution of the completers by country and region is given in Table 2 below.

Table 2: Number of completers by country and region

Country	Region	No. recruited	Completers
Belgium	Mol	185	100
Romania	Bucharest	204	100
	Giurgiu	127	100
Slovakia	Bratislava	118	100
	Stara Lubovna	154	100

3 Data description

The responses of primary interest are allergies in children at 18months of age. This was measured by atopic eczema in child, other dermatitis of allergy in child, specific IgE for inhalant allergies in child and specific IgE for food allergies in child. All measures are binary.

Variables collected in the study were categorized into Biomarkers of exposure (Pollutants) and Biomarkers of effect. A pictorial representation of the objectives of the study with respect to these sets of variables is shown in Figure 1.

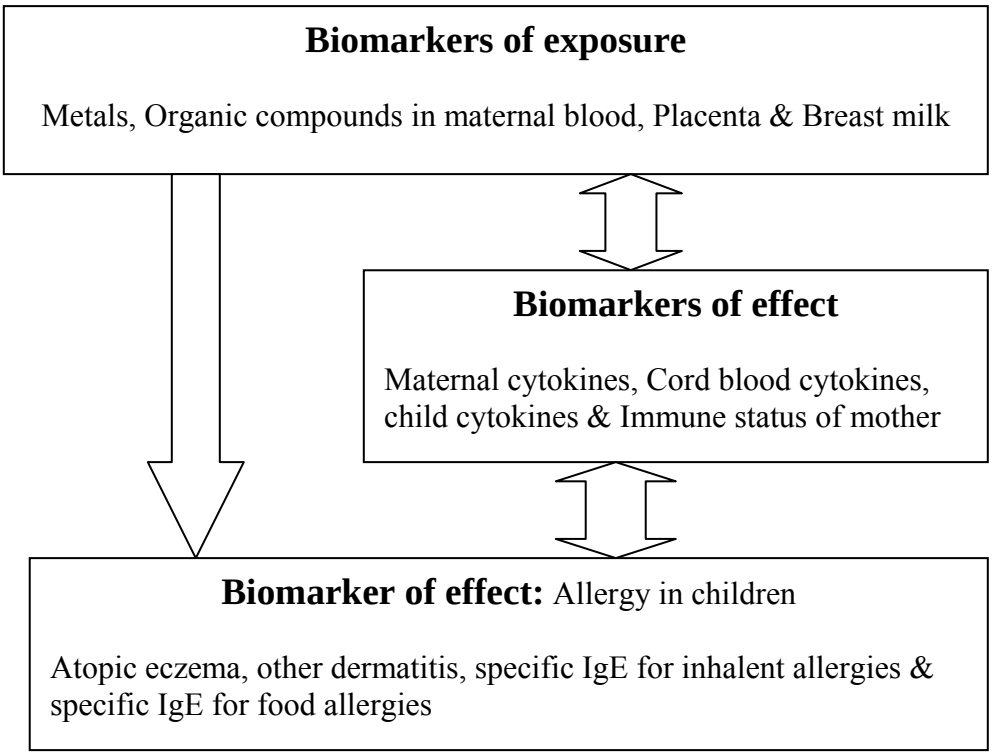

Figure 1: Biomarkers of exposure and Biomarkers of effect

3.1 Biomarkers of exposure

Biomarkers of exposure comprised of inorganic and organic compounds. Inorganic compounds consisted of metals and were measured in samples of maternal blood and placenta. Metals included Lead and Cadmium. Organic compounds consisted of Chlorinated benzenes, Organochlorine insecticides, and Polychlorinated biphenyls (PCBs) and were measured in samples of maternal blood, placenta and breast milk. All biomarkers of exposure are continuous variables. Tables A1.1 and A1.2 in Appendix A describe inorganic and organic compounds analysed in the biological samples. All biomarkers of exposure were covariates of interest in the evaluation of environmental chemicals relating to allergy in child hood years.

In practice (Noakes et al., (2006)), measurements from organic compounds with similar chemical structural formulae are summed. The summation is performed within groups as specified in Tables

A1.1 and A1.2 in Appendix A: maternal blood serum, placenta and breast milk. However, the magnitudes of the (measurements) values of the various organic compounds in a given group vary widely. In order to ensure that those compounds with higher magnitudes do not run the analysis, the values of each compound were standardized, thereby assigning equal weights to all variables contributing to the sums.

In total, there were nine sums of organic compounds: sum of Chlorinated benzenes in maternal blood serum *(MBS-sum1)*, sum of Polychlorinated biphenyls (PCBs) in maternal blood serum *(MBS-sum2)*, sum of Organochlorine insecticides in maternal blood serum*(MBS-sum3)* ,sum of Chlorinated benzenes in placenta *(PLAT-sum1)*, sum of Polychlorinated biphenyls (PCBs) in placenta*(PLAT-sum2)* , sum of Organochlorine insecticides in placenta *(PLAT-sum3)*, sum of Chlorinated benzenes in breast milk *(BM-sum1)*, sum of Polychlorinated biphenyls (PCBs) in breast milk*(BM-sum2)* ,and sum of Organochlorine insecticides in breast milk *(BM-sum3)*. These sums were considered for analyses of organic compounds.

3.2 Biomarkers of effect

Biomarkers of effect consisted of maternal peripheral blood cytokines, cord blood cytokines, placenta cytokines, child blood cytokines, atopic eczema in child, other dermatitis of allergy in child, specific IgE for inhalant allergies in child, specific IgE for food allergies in child and immune status of the mother. The immune status of the mother was measured by her allergic history, total IgE in maternal and cord blood and atopic or nonatopic status of the mother.

These variables, including weight and length are continuous while the remaining were categorical (Table 3). A complete list of biomarkers of effect is given in Appendix A (Table A2.1).

Table 3: Categorical biomarkers of effect

Biomarker	Categories
Atopic eczema in child	0 = no, 1 = yes
other dermatitis in child	0 = no, 1 = yes
Specific IgE for inhalant allergies in child	0 = no, 1 = yes
Specific IgE for food allergies in child	0 = no, 1 = yes
Allergic history of the mother	0 = no, 1 = yes
Specific IgE (Phadiatop) in maternal blood	0<0.35kU/l, 1 >=0.35kU
Atopic or nonatopic status of the mother	0 = no, 1 = yes , 3 = doubtful
Low level cord blood IgE	0<0.35kU/l, 1>=0.35kU/l

Among cord blood cytokines, only INFgphacord, il4phacord, il5phacord, il10phacord and il12phacord are of interest since stimulation was low for the other cord blood cytokines. Stimulation refers to causing cells to multiply and can be done using different agents. Agents used for stimulation in this study included PHA (Phytohemagglutinin), lactalbumin, ovalbumin and house dust mite. PHA is a plant product and induces division white blood cells and lactalbumin is cows' milk protein. Only white blood cells of individuals who are allergic to these compounds will divide.

Summary of the objectives of the study

Participants were recruited from three countries: Slovakia, Romania and Belgium. Five regions were selected from the three countries: Slovakia (Bratislava, Stara Lubovna), Romania (Bucharest,

Giurgiu) and Belgium (Mol). Altogether, there were 500 mothers and 500 children from the three countries.

In this study, we will investigate the effect of neonatal (cord blood) cytokines, maternal cytokines and biomarkers of exposure on the development of allergic outcomes in children at 18 months of age. Moreover, the effect of biomarkers of exposure on immune status of the mother, cord blood cytokines, placenta cytokines and child cytokines will be examined. As a final step, the predictive ability of neonatal cytokines and maternal cytokines on the development of allergic outcomes in children at 18 months of age will be evaluated.

4 Statistical methodology

In this section we briefly present various statistical methods used to analyze the data inorder to draw conclusions based on the different objectives of the study. Techniques used to explore the data are described in section 4.1, and the regression techniques are concisely described in the subsequent subsections.

4.1 Exploratory data analysis

Spearman rank correlation was used to capture the association between pairs of continuous variables while Fisher's exact test was used for associations among categorical variables. Fisher's exact test was used since frequencies were less than 5 in some cells.

4.2 Regression techniques

In this section regression techniques used to analyze the data are described. They include Multiple Regression analysis, Multiple Logistic Regression analysis and Multivariate Logistic Regression analysis. In addition, model building strategies used to identify subsets of explanatory variables are presented. They include the Stepwise, Forward and adjusted R-square selection procedures. The selected explanatory variables from these strategies were considered in the Multivariate Logistic Regression analysis.

4.2.1 Multiple regression analysis

For continuous responses, Multiple Regression analysis was done to select variables explaining the response and the fitted models were of the form below.

$$Y_i = \beta_o + \beta_1 X_{i1} + ... + \beta_{p-1} X_{i,p-1} + \varepsilon_i ,$$

where Y_i is the value of the response variable for the i^{th} observation, $\beta_o, \beta_1, ..., \beta_{p-1}$ are the parameters of the model, $X_{i1}, X_{i2}, ..., X_{i,p-1}$ are known and constant values of the explanatory variables for the i^{th} observation, ε_i is the error term which is assumed to be normally distributed with the mean zero and variance σ^2.

This is a first-order regression model with *p-1* explanatory variables whose effects on the mean response are additive, that is, the parameter β_1 represents a change in the mean response $E\{Y\}$ per unit increase in X_1 when $X_2, \ldots, X_{P-1}$ are held constant. This regression model encompasses both qualitative and quantitative explanatory variables (Kutner *et al*., 5th edition 2005).

4.2.2 Multiple logistic regression analysis

In order to study the association between allergy outcomes and the biomarkers, multiple logistic regression models were conducted for binary responses. The following model is considered for logistic regression at values $X = (x_1, x_2, ..., x_p)$ of p explanatory variables.

$$Logit\ [P(Allergy\ outcome=1)] = \beta_o + \beta_1 x_1 + \beta_2 x_2 + ... + \beta_p x_p$$

The parameter β_i refers to the effect of x_i on the log odds that *Allergy outcome =1*, controlling the other explanatory variables. For example, exp(β_i) is the multiplicative effect on the odds of a 1-unit increase in x_i , at fixed levels of other explanatory variables (Agresti., (2002)).

4.2.3 Model building strategies

The adjusted R-squared ($R_{a,p}^2$, *p* being number of parameters in the regression model), Stepwise and Forward procedures were used to select the subset of explanatory variables that were included in Multivariate Logistic Regression analysis.

The $R_{a,p}^2$ was considered to obtain the proportion of variance in the response variable explained by the explanatory variables. Also, $R_{a,p}^2$ takes into account the number of explanatory variables in the model through the degrees of freedom. A set of explanatory variables for which $R_{a,p}^2$ is maximum.

The Stepwise procedure develops a sequence of regression models, at each step adding or deleting an explanatory variable. For multiple linear regression, variables are added or deleted depending on the t-statistic associated with a specific estimated regression parameter and its P-value. Incase of logistic regression, the decision is based on the Wald statistic for a particular estimated regression parameter and its P-value. Forward selection procedure adds an explanatory variable if the P-value associated with its Wald statistic is less than a specified significance level.

All Variables selected by the different selection procedures will be considered in the multivariate logistic regression analysis and model reduction will be done using the Wald test.

4.2.4 Multivariate logistic regression analysis

Since four response variables were measured on each subject, multivariate logistic regression models, which capture the association between observations from the same subject, were fitted. Observations from the same subject were expected to be more alike hence correlated whereas observations from different subjects were assumed to be independent.

Let Y_i denote the vector of observed responses for the i^{th} subject and μ_i be the vector of mean responses for the i^{th} subject. Y_i is the binary response taking values of 0 (if subject never had allergy) and 1 (if subject had allergy). Let x_{ij} denote the value of explanatory variables *j* (*j* = 1, 2,…, *p*) for subject *i*. Then, $\eta_i = \sum_j \beta_j x_{ij}$, *i* = 1, 2,……., N., is a linear combination of explanatory variables, β_j being a vector of regression parameters for the explanatory variables. This linear combination is called the linear predictor. If we let $\mu_i = E(Y_i)$, *i* = 1,……,N., the model links μ_i to η_i by $\eta_i = g(\mu_i)$, where g is link function. The link function g links μ_i to explanatory variables through the formula $g(\mu_i) = \eta_i = \sum_j \beta_j x_{ij}$, *i* = 1, 2,……., N. For binary data, the mean translates

into success probabilities; $\mu_{ij} = \pi_{ij} = P(Y_{ij} = 1)$, with v(μ_{ij}) = $\pi_{ij}(1-\pi_{ij})$ and the link function is the logit link defined as logit (μ_{ij}) = In [π_{ij} /(1 – π_{ij})], (Agresti., (2002)).

All confirmatory statistical analysis was carried out in SAS version 9.1 and R version 2.2.0 at 5% significant level. The 5% significant level was chosen since it is consistent with the 95% confidence interval estimation commonly used in practice.

4.2.5 Accounting for missingness

There were missing observations in the biomarkers of exposure and biomarkers of effect. To account for the missing observations, multiple imputations were done for missing observations using Markov chain Monte Carlo (MCMC) method and multivariate logistic regression models described in Section 4.2.4 were fitted on complete case data and imputed data sets. Results from these models will be presented. Parameter estimates from imputed datasets were combined and standard errors of the combined estimates were estimated. For *M* imputations, the estimate of the parameter β is $\hat{\beta}^{*} = (\sum_{m=1}^{M} \hat{\beta}^{m})/M$ and its standard error is $\sqrt{W + ((M+1)/M) * B}$, W and B being within and between imputation variances given by the following expressions:

$B = [\sum_{m=1}^{M} (\hat{\beta}^{m} - \hat{\beta}^{*})(\hat{\beta}^{m} - \hat{\beta}^{*})']/M - 1$ and $W = (\sum_{m=1}^{M} U^{m})/M$, $U = \hat{\text{var}}(\hat{\beta})$, (Molenberghs and Verbeke (2005)).

4.2.6 Goodness of fit tests and diagnostics for statistical models

To check for goodness of fit of multiple logistic regression models, Hosmer and Lemeshow test was used. This test places subjects into deciles based on the model predicted probabilities, then computes a Pearson chi-square test based on the observed and expected number of subjects in the deciles. The statistic is compared to a chi-square distribution with *t* degrees of freedom, *t* being the number of decile groups minus 2 (Agresti., (2002)).

Diagnostic residual plots

Diagnostics of the multivariate logistic models were done using deviance and Pearson studentized residuals against the predicted probabilities plots. To check the adequacy of the models, the Lowess method (locally weighted scatter plot smoothing) was employed. The lowess method is a refined non-parametric method which obtains a smooth curve by fitting successive regression functions in the neighbourhood. Under this procedure if the model is adequate then the expectation of the difference between the observed and the predicted probabilities should be zero, therefore a lowess smooth curve should be a horizontal line with zero intercept. The deviance and Pearson residuals were used for the diagnostics. The Pearson residuals are given by $r = \frac{(Y_i - \hat{\pi}_i)}{\sqrt{\hat{\pi}_i(1-\hat{\pi}_i)}}$, where Y_i is the response and $\hat{\pi}_i$ is the estimated probability. When the Pearson residuals are divided by their standard deviation, we get the studentized Pearson residuals. The Deviance residuals are given by

$$dev_i = sign(Y_i - \hat{\pi}_i)\sqrt{-2[Y_i \log_e(\hat{\pi}_i) + (1-Y_i)\log_e(1-\hat{\pi}_i)]},$$

where the sign is positive when $Y_i \geq \hat{\pi}_i$ and negative when $Y_i < \hat{\pi}_i$. The residual plots provide some information about the adequacy of the logistic regression fit. The residuals are plotted against the predicted probabilities using the Lowess method (Kutner *et al*., 5th edition 2005).

4.3 Prediction techniques

Reciever Operating Characteristic (ROC) curves were used to determine whether cord blood and maternal cytokines are predictive biomarkers of allergic outcomes in children at 18 months of age. In addition, the predictive ability of pollutants was assessed. A ROC curve is a graphical plot of the sensitivity vs. (1 - specificity) for a binary classifier system as its discrimination threshold is varied. The ROC curve can also be represented equivalently by plotting the fraction of true positives vs. the fraction of false positives. The accuracy of prediction is measured by the area under the ROC curve. The area under the ROC curve is interpreted as probability that when we randomly pick one positive and one negative example, the classifier will assign a higher probability of being positive example than to the negative. An area of 1 represents a perfect prediction; an area close to 1 represents good prediction; an area of 0.5 lies at the boundary of the random guess and an area less than 0.5 is worse than guessing the outcome. The formula used to calculate the variance, σ_A^2, of the area under the curve is given by the expression;

$$\sigma_A^2 = (A(1-A) + (m-1)(P_{xxy} - A^2) + (n-1)(P_{xyy} - A^2))/mn,$$
$$P_{xxy} = A/(2-A) \text{ and } P_{xyy} = 2A^2/(1+A),$$

where A is the area under the curve, m is the number of positive examples and n is the number of negative examples. Further, confidence intervals for the areas under the curve were obtained. A confidence interval containing 0.5 was considered non informative. The ROCR package version 1.0-2 was used to draw ROC curves (Sing., *et al* (2007)).

5 Results

In this section, results obtained from exploratory data analysis and statistical models are presented. Exploratory data analysis is presented first and subsequently analysis from statistical models will follow.

5.1 Exploratory data analysis

The data was explored through the use of summary statistics of each variable, which characterizes the distributional properties of these variables, association between variables and the degree of missingness in the data.

Spearman rank correlation was used to capture the association between pairs of continuous variables while Fisher's exact test was used to capture associations among categorical variables. Fisher's exact test was used since frequencies were less than 5 in some cell counts of the 2 by 2 tables. Exploratory data analysis of biomarkers of exposure is presented in section 5.1.1 and that of biomarkers of effect in section 5.1.2.

5.1.1 Biomarkers of exposure

Biomarkers of exposure comprised of inorganic and organic compounds. Inorganic compounds were metals, which included Lead in maternal blood serum (Pbblood), Lead in placenta (Pbplacenta) Cadmium in maternal blood serum (Cdblood) and Cadmium in placenta (Cdplacenta).

The highest percentage (43.2%) of missing observations was observed in breast milk samples while the smallest (0.2%) was observed in placental organic compounds. Figure 2 illustrates proportions of missing observations in biomarkers of exposure variables. The proportions of missing observations in 27 variables were as low as <.05, <.06 in 23 variables and proportions were moderate (0.43) in other 23 variables. Table B1 in Appendix B contains information about missing observations of each biomarker of exposure.

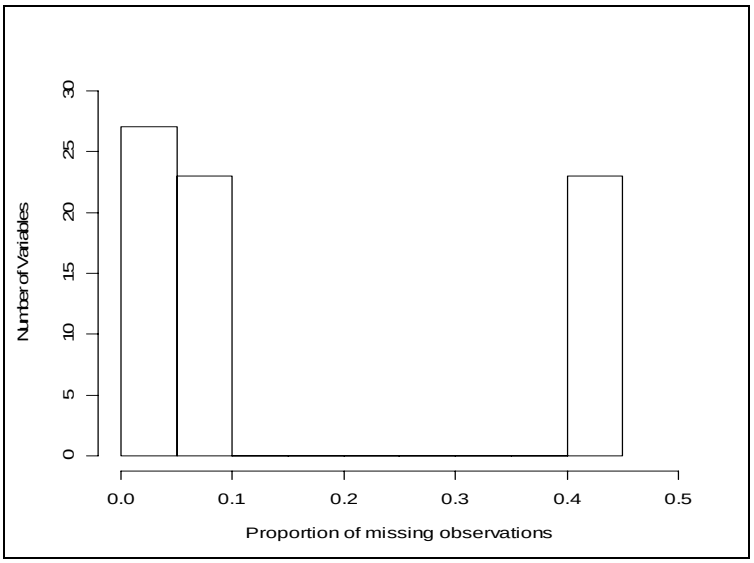

Figure 2: Proportion of missing observations in Biomarkers of exposure

Since more than 50% of the information for all biomarkers of exposure is available, all biomarkers will be considered for further analysis. Multiple imputations will be employed for missing observations using the MCMC method.

Inorganic Compounds

In this section, summary statistics and correlations between pairs of the metals in the biomarkers of exposure are presented by region. A brief description of summary statistics over all countries and by country is then given and details are provided in Tables B3, B5 and B6 in Appendix B. Table 4 below summarizes concentrations of Lead and Cadmium in maternal blood and placenta samples by region.

Table 4: Summary of concentrations of metals by region (µg/l)

Country (Region)	Maternal blood				Placenta			
	Pbblood		Cdblood		Pbplacenta		Cdplacenta	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Slovakia								
Brastislava	22.563	6.706	0.255	0.244	0.014	0.006	0.007	0.009
Stara Lubovna	26.543	11.9	0.277	0.199	0.017	0.015	0.008	0.004
Romania								
Bucharest	43.042	15.882	0.795	2.042	0.027	0.016	0.008	0.004
Giurgiu	56.858	34.091	0.803	1.203	0.083	0.148	0.010	0.005
Belgium								
Mol	19.774	8.270	0.367	0.182	0.014	0.011	0.009	0.010

Lead concentrations were higher in maternal blood and placenta samples in all the countries than Cadmium concentrations (Table B5 in Appendix B). Widest ranges of Lead concentrations in maternal blood serum (Pbblood) and placenta samples (Pbplacenta) were observed in Romania. Slovakia had widest ranges of Cadmium concentrations in placenta samples (Cdplacenta) (Table B6, Appendix B).

The highest concentrations of both metals on average were found in biological samples from Giurgiu region in Romania. Lowest Lead concentrations were found in Mol (Belgium). On average all regions had similar Cadmium concentrations in placenta (Table 4). From Table B7 in Appendix B, Giurgiu had widest ranges of Lead concentrations in maternal blood serum (Pbblood) and placenta samples (Pbplacenta). Bucharest had widest ranges of Cadmium concentrations in maternal blood serum (Cdblood) and Brastislava had widest ranges of Cadmium concentrations in placenta samples (Cdplacenta).

Table B3 in Appendix B presents results of overall concentration of Lead and Cadmium in maternal blood serum and placenta samples. Generally, Lead had higher concentrations in both samples than Cadmium on average. Concentrations of both metals were very low in placenta samples. Of course, the baby only gets a fraction of what the mother has, thus, concentrations of metals in placenta samples are quite small in magnitude compared to those of the mother.

Positive correlations were observed between Cadmium concentrations in placenta and maternal blood samples (0.547, P-value <.0001) and also between Lead concentrations in placenta and maternal blood (0.641, P-value <.0001). These correlations imply that Cadmium in placenta and maternal blood samples and Lead concentrations in placenta and maternal blood samples are

related. This makes sense as babies get blood from their mothers, hence, if the mother has high Lead concentration, it is likely that the baby's placenta blood will have relatively high concentrations of Lead. Details are shown in Table B4 below in Appendix B.

Organic Compounds

Concentrations of organic compounds were determined in maternal blood, placenta and breast milk samples. Sums of structurally similar organic compounds were considered for analyses of organic compounds. In this section, summary statistics of these sums over all countries, by country and by region are presented.

Concentrations of organic compounds were on average high (>180 ng/g fat) in all samples over all countries although that of organochlorine insecticides in breast milk (BM-sum3) was extreme (2311 ng/g fat) (Table B8 in Appendix B).

Table B9 in Appendix B provides correlations between pairs of sums of organic compounds over all the countries. Six correlation estimates are greater than 0.5 in magnitude and are significantly different from zero. The correlation estimate occurred between sum of organochlorine insecticides in breast milk (BM-sum3) and sum of organochlorine insecticides in placenta (PLAT-sum3) (0.906, P-value <.001). Although correlations amongst other sums were significant, their magnitudes were less than 0.5.

Concentrations of organic compounds in maternal blood, placenta and breast milk samples were further analysed by country (Table B10 in Appendix B). The highest maternal blood (MBS-sum3), placenta (PLAT-sum3) and breast milk (BM-sum3) concentrations of the total sum of organochlorine insecticides were found in Romania. Belgium had highest concentrations of the total sum of Chlorinated benzenes (MBS-sum1) and total sum of PCBs (MBS-sum2) in maternal blood samples as well as lowest concentrations of the total sum of Chlorinated benzenes (MB-sum1) and total sum of PCBs (MB-sum2) in breast milk samples. Highest concentrations of the total sum of Chlorinated benzenes (PLAT-sum1) and total sum of PCBs (PLAT-sum2) in placenta samples were observed in Slovakia.

Table B11 in Appendix B shows ranges of concentrations of organic compounds by country. Among maternal blood serum sums (MBS-sum1, MBS-sum2, MBS-sum3), Belgium had the widest range of the total sum of Chlorinated benzenes (MBS-sum1). Slovakia had the widest range of the total sum of organochlorine insecticides (PLAT-sum3) in placenta samples. Romania had the widest range of the total sum of organochlorine insecticides (BM-sum3) in breast milk samples.

Correlations between pairs of sums of organic compounds were also studied in each country. Correlation estimates from Slovakia (Table B12 in Appendix B) are presented first, then Romania (Table B13 in Appendix B) and at last Belgium (Table B14 in Appendix B).

As can be seen in Tables 5a and 5b, highest concentrations of the total sum of Chlorinated benzenes (MBS-sum1) and total sum of organochlorine insecticides (MBS-sum3) in maternal blood serum were found in Giurgiu in Romania. On the other hand, highest concentrations of the total sum of PCBs (MBS-sum2) in maternal blood samples were observed in Mol in Belgium. Ranges of concentrations of organic compounds by region are provided in Tables B14a and B14b in Appendix B.

Table 5a: Concentrations of organic compounds by region (ng/g fat)

Variable	Romania				Belgium	
	Bucharest		Giurgiu		Mol	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
MBS-sum1	147.115	217.375	489.936	1066	401.111	1526
MBS-sum2	117.998	182.749	111.370	136.919	297.108	211.529
MBS-sum3	1375	1525	1683	1570	447.501	350.923
PLAT-sum1	308.285	726.138	181.630	143.776	63.962	108.739
PLAT-sum2	108.981	111.966	128.140	141.313	104.742	87.3111
PLAT-sum3	1295	871.589	1729	1275	137.432	123.327
BM-sum1	206.721	187.177	220.320	182.102	98.090	188.444
BM-sum2	285.466	177.589	358.556	398.183	163.770	87.799
BM-sum3	4218	3209	4888	2771	448.754	449.027

Table 5b: Concentrations of organic compounds by region (ng/g fat)

Variable	Slovakia			
	Brastislava		Stara Lubovna	
	Mean	Std Dev	Mean	Std Dev
MBS-sum1	287.654	1170	215.841	187.333
MBS-sum2	207.694	168.483	241.237	221.179
MBS-sum3	321.079	267.178	277.949	190.643
PLAT-sum1	410.139	2146	192.505	150.776
PLAT-sum2	693.618	4947	160.089	123.219
PLAT-sum3	756.565	4124	211.371	140.279
BM-sum1	178.348	193.549	225.829	186.719
BM-sum2	301.740	195.765	291.350	182.614
BM-sum3	780.598	525.291	600.928	287.371

5.1.2 Biomarkers of effect

In this section, we present the degree of missingness, summary statistics and correlations between pairs of biomarkers of effect. Biomarkers of effect comprised of maternal peripheral blood cytokines, cord blood cytokines, placenta cytokines and child blood cytokines. In addition, summary statistics from allergies of child at 18months and immune status of the mother are briefly described.

Measurements for all the four groups of cytokines were taken in Slovakia. In Belgium, apart from placenta cytokines for which samples were of bad quality, measurements for the other three groups of cytokines were available. Only measurements from placenta cytokines were provided from Romania. Measurements of maternal peripheral blood cytokines, cord blood cytokines and child blood cytokines in Romania could not be obtained because the quality of samples was bad. This implies that, in the analysis of the associations between cord blood cytokines and allergies and associations between maternal cytokines and allergies, Romania will not be considered. In addition, Romania will not be considered for the relationships between child cytokines and pollutants and cord blood cytokines and pollutants. Belgium will also not be considered for relationships between placenta cytokines and pollutants.

Although more than 50% of the observations were missing in maternal peripheral blood, cord blood, placenta and child blood cytokines, these cytokines will be included in statistical analysis as they are the responses of the secondary objective of the study. Multiple imputations using the MCMC method will be employed for missing observations. Multiple imputations for cytokines will only be employed for countries in which the cytokines were collected.

Figure 3 below presents a summary of missing observations of all biomarkers of effect. Five variables had highest proportions of missing observations (> 0.80). More details are given in Table B2 in Appendix B.

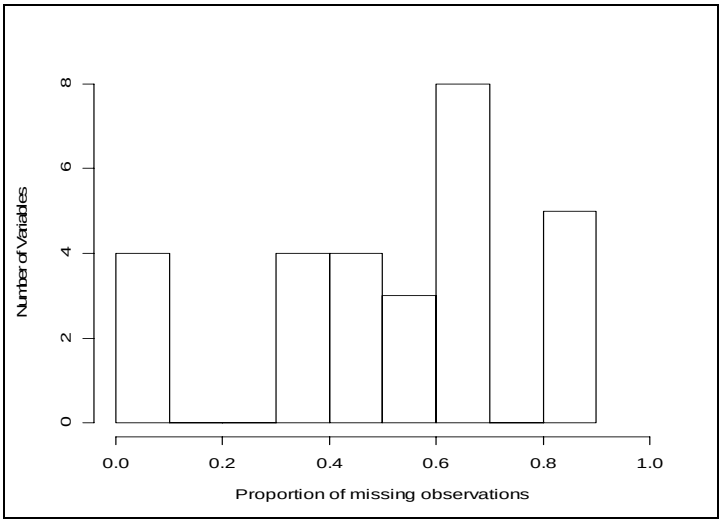

Figure 3: Proportion of missing observations in Biomarkers of effect

Allergies of children at 18months of age

Allergies are our responses of primary interest. They included Atopiceczema, Otherdermatitis (Otherdet), Specific IgE for inhalant allergies (PhadCHinhalant) and Specific IgE for food allergies (fx5Chfood). At 18moths, nearly 30% of the children had missing observations on atopic eczema, other dermatitis, specific IgE for inhalant allergies in child blood and specific IgE for food allergies in child blood.

All measures of allergies were binary and Fisher's exact test was used to search for associations between them. The Fisher's exact test revealed that there was no significant association between pairs of allergic outcomes (Table 6). These results indicate that these outcomes may not be modeled multivariately although they were collected from the same individuals. However, lack of significant associations between pairs of allergic outcomes in the study population may be due to the zero counts in some cells of 2 x 2 tables. For example, there were no children with both Atopic eczema and Specific IgE for inhalant allergies, Otherdermatitis and Specific IgE for inhalant allergies and, Specific IgE for inhalant allergies and Specific IgE for food allergies. Nevertheless, such cases can exist in practice. Thus, multivariate models will be considered.

Table 6: Associations between allergic outcomes outcomes

Pair of allergy measure	P-value
Atopiceczema and Otherdet	1.00
Atopiceczema and PhadCHinhalant	1.00
Atopiceczema and fx5Chfood	1.00
Otherdet and PhadCHinhalant	1.00
Otherdet and fx5Chfood	0.56
PhadCHinhalant and fx5Chfood	1.00

Maternal peripheral blood cytokines

The percentage of missing observations among maternal cytokines ranged from 41% to 62%. Summary statistics of maternal peripheral blood cytokines are presented in Table 7. Cytokine INFgphamaternal was observed to have highest concentration in maternal blood cytokines while lowest concentrations were found in il4phamaternal.

Table 7: Summary statistics of maternal peripheral cytokines (pg/ml)

Variable	Mean	Std Dev	Min	Max
INFgphamaternal	17611	28237	2.00	202749
il4phamaternal	27.951	43.457	0.10	220.1
il5phamaternal	93.709	144.198	0.55	578.2
il10phamaternal	233.835	282.566	0.50	1320
il12phamaternal	297.62	388.008	1.00	1476

All maternal blood cytokines were highly and significantly correlated, with highest magnitude (0.869) between il12pha and INFgpha and the smallest (0.685) between il10pha and INFgpha (Table B15 in Appendix B). This indicates that multi-collinearity may occur and therefore should be investigated in model building

Cord blood cytokines

The number of missing observations among cord blood cytokines ranged from 46% to 63% (Table B2 in Appendix B). The largest correlation estimate in magnitude (0.833) was between IFNgphacord and il10phacord whereas the smallest (0.597) was between il12phacord and il4phacord (Table B16 in Appendix B). In Table 8, we observe that highest and lowest concentrations among Cord blood cytokines were found in INFgphacord and il4phacord respectively.

Table 8: Summary statistics of cord blood cytokines (pg/ml)

Variable	Mean	Std Dev	Min	Max
il4phacord	5.636	8.948	0.1	66.3
il5phacord	13.303	23.307	0.1	215.4
il10phacord	207.853	299.434	0.5	1703
il12phacord	191.823	341.296	1.0	2989
IFNgphacord	417.058	549.335	2.0	2291

Placenta cytokines

All placenta cytokines had over 65% missing observations. Cytokines il10placenta and tnf had the largest correlation coefficient in magnitude (0.641, P-value = <.0001) and correlation coefficients between pairs of other placenta cytokines were smaller (0.5) in magnitude (Table B18 in Appendix

B). Cytokines il5placenta and il4placenta had highest and lowest concentrations in placenta respectively (Table 9).

Table 9: Summary statistics of placenta cytokines (pg/ml)

Variable	Mean	Std Dev	Min	Max
INFgplacenta	2.742	2.358	1.035	23.079
il4placenta	0.686	1.435	0.194	13.355
il5placenta	8.621	26.588	0.75	199.036
il10placenta	7.204	17.201	0.214	174.209
tnfplacenta	7.275	17.451	0.393	166.369

Child blood cytokines

All child cytokines had over 84% of missing observations. Except il4 and il10 and il4 and INFg, correlation estimates between remaining pairs of cytokines are large in magnitude and significant (>0.5) Table B17 in Appendix B. Cytokine il4child was the least concentrated while il2child was the most concentrated in the blood of children (Table 10).

Table 10: Summary statistics of child blood cytokines (pg/ml)

Variable	Mean	Std Dev	Min	Max
INFgchild	680.955	837.344	2.5	4437
il2child	2060	1839	3.0	7872
il4child	7.818	6.094	2.5	34.39
il5child	274.898	357.922	1.5	1646
il10child	212.355	189.709	2.5	1007

Frequencies of children with allergies were obtained by region (Table 11). Mol (Belgium) had the highest prevalence of Atopiceczema (32.7%). About 9% of children had Otherdermatitis (Otherdet) in Bratislava (Slovakia) and no child had Otherdermatitis in Stara Lubovna (Slovakia). Three children in all regions had specific IgE for inhalant allergies in child hood (PhadCHinhalant). The highest prevalence (32.7%) of Atopic-eczema among children was observed in Belgium (Table B20 in Appendix B). About 15.6% of children in all countries had Atopic eczema at 18months of age (Table B19 in Appendix B).

Table 11: Number of children (%) with allergy measures by region

Measure of Allergy	Levels	Country (Region)				
		Slovakia		Romania		Belgium
		Bratislava	Stara Lubovna	Bucharest	Giurgiu	Mol
Atopiceczema	No	72 (82.8)	78 (89.7)	49 (86.0)	56 (93.3)	37 (67.3)
	Yes	15 (17.2)	9 (10.3)	8 (14.0)	4 (6.7)	18 (32.7)
Otherdet	No	79 (90.8)	87 (100)	53 (93.0)	57 (95.0)	53 (96.4)
	Yes	8 (9.2)	0	4 (7.0)	3 (5.0)	2 (3.6)
PhadCHinhalant	No	85 (100)	86 (100)	47 (100)	39 (95.1)	71 (98.6)
	Yes	0	0	0	2 (4.9)	1 (1.4)
fx5Chfood	No	78 (91.8)	82 (95.4)	47 (97.9)	40 (97. 6)	68 (94.4)
	Yes	7 (8.2)	4 (4.6)	1 (2.1)	1 (2.4)	4 (5.6)

Immune status of the mother

Immune status of the mother was measured by allergic history of the mother, total IgE in maternal and cord blood and atopic or nonatopic status of the mother. Lowest percentage of missing observations was observed from immune status of mothers in which all variables had less than 0.3% missing observations. The highest percentage (71.0%) of allergic mothers and atopic mothers (36.4%) was observed in Mol region in Belgium (Table 12).

Table 12: Number of mothers (%) by region

Measure of immunity	Levels	Country (Region) Slovakia Bratislava	Slovakia Stara Lubovna	Romania Bucharest	Romania Giurgiu	Belgium Mol
Allergic mother	No	33 (33.3)	61 (61.0)	59 (59.0)	64 (64.0)	29 (29.0)
	Yes	66 (66.7)	39 (39.0)	41 (41.0)	36 (36.0)	71 (71.0)
Atopic_nonAtopic	No	27 (27.3)	32 (32.3)	37 (38.5)	32 (33.0)	19 (19.2)
	Yes	29 (29.3)	11 (11.1)	11 (11.5)	11 (11.3)	36 (36.4)
	Doubtful	43 (43.4)	56 (56.6)	48 (50.0)	54 (55.7)	44 (44.4)

Belgium had the highest percentage (71.0%) of the allergic mothers at the same time the highest percentage (36.4%) of atopic mothers. Romania had the lowest proportion of allergic (38.5%) and atopic (11.4) mothers (Table B22 in Appendix B). Table 13 shows that magnitudes of correlation estimates among maternal immunity measures were small (<0.5) which suggests absence of multi-collinearity among variables. All variables will be included in statistical analysis.

Table 13: Correlations between maternal immunity measures over all countries

Variables	Allergicmother	Atopic/nonAtopic	TotalIgE
Allergicmother	1		
Atopic/nonAtopic	0.431 *(<.0001)*	1	
TotalIgE	0.038 *(0.400)*	0.275 *(<.0001)*	1

Numbers of mothers with allergies over all countries are given in Table B21 in Appendix B. About 51% of the mothers were allergic in the past and 20% were atopic. Romania had highest concentrations of TotalIgE (503.308 kU/l) in maternal and cord blood samples with Giurgiu region having the highest concentrations (727.414 kU/l) among all regions (Table B23 in Appendix B).

Correlations between allergic outcomes (Responses) and explanatory variables.

In this section Spearman rank correlation coefficients between allergic outcomes and explanatory variables are done inorder to have an idea of which variables might explain the responses. Correlations have been based on the primary objectives of the study and are presented in the following order: allergic outcomes and cord blood cytokines, allergic outcomes and maternal cytokines and allergic outcomes and pollutants (biomarkers of exposure).

Table 14: Correlations *(P-values)* between allergic outcomes and cord blood cytokines

Variable	Atopiceczema	Otherdet	PhadCHinhalant	fx5Chfood
il4phacord	0.283*(0.001)*	0.102*(0.175)*	0.050*(0.491)*	0.0757*(0.299)*
il5phacord	0.152*(0.029)*	0.112*(0.107)*	0.024*(0.726)*	-0.004*(0.954)*
il10phacord	0.219*(0.004)*	0.078*(0.318)*	0.092*(0.226)*	-0.003*(0.972)*
il12phacord	0.021*(0.807)*	0.0791*(0.360)*	0.129*(0.119)*	0.042*(0.613)*
IFNgphacord	0.223*(0.003)*	0.115*(0.129)*	0.097*(0.186)*	-0.010*(0.888)*

All correlation estimates between allergic outcomes and cord blood cytokines are very small in magnitude (Table 14), an indication that none of the cord blood cytokines might explain the probability of allergy outcomes. Similarly, magnitudes of correlation estimates between allergic outcomes and maternal cytokines are too small. Maternal cytokines may also not contribute to the probability of allergy outcomes (Table 15).

Table 15: Correlations between allergic outcomes and maternal cytokines

	Atopiceczema	Otherdet	PhadCHinhalant	fx5Chfood
INFgphamaternal	0.228*(<.001)*	0.134*(0.053)*	0.042*(0.531)*	-0.038*(0.572)*
il4phamaternal	0.150*(0.027)*	0.144*(0.034)*	0.106*(0.110)*	-0.046*(0.489)*
il5phamaternal	0.196*(0.003)*	0.149*(0.025)*	0.077*(0.239)*	-0.013*(0.843)*
il10phamaternal	0.109*(0.203)*	0.179*(0.036)*	-0.016*(0.841)*	-0.047*(0.569)*
il12phamaternal	0.217*(0.014)*	0.093*(0.296)*	0.016*(0.853)*	-0.109*(0.195)*

Table 16 reveals that estimates of correlations between allergic outcomes and pollutants are small in magnitude, which suggests that pollutants may not be related to allergy outcomes. Irrespective of the small magnitudes of correlations between allergies and explanatory variables, relationships between allergies and these explanatory variables will be investigated using statistical models.

Table 16: Correlations *(P-values)* between allergic outcomes and pollutants

Variables	Atopiceczema	Otherdet	PhadCHinhalant	fx5Chfood
MBS-sum1	-0.033 *(0.554)*	-0.137 *(0.013)*	0.041 *(0.467)*	0.002 *(0.973)*
MBS-sum2	-0.014 *(0.801)*	-0.051 *(0.356)*	-0.018 *(0.757)*	-0.079 *(0.165)*
MBS-sum3	0.071 *(0.198)*	-0.007 *(0.896)*	-0.038 *(0.499)*	-0.094 *(0.098)*
PLAT-sum1	-0.061 *(0.259)*	-0.066 *(0.220)*	0.136 *(0.013)*	-0.030 *(0.591)*
PLAT-sum2	-0.125 *(0.020)*	-0.029 *(0.591)*	0.042 *(0.446)*	0.010 *(0.851)*
PLAT-sum3	-0.124 *(0.022)*	0.016 *(0.767)*	-0.032 *(0.562)*	-0.011 *(0.847)*
BM-sum1	-0.153 *(0.030)*	-0.088 *(0.211)*	0.100 *(0.169)*	-0.202 *(0.005)*
BM-sum2	0.000 *(0.995)*	-0.061 *(0.385)*	-0.034 *(0.639)*	-0.021 *(0.774)*
BM-sum3	0.102 *(0.149)*	0.011 *(0.878)*	0.091 *(0.212)*	-0.077 *(0.291)*

5.2 Statistical models

Statistical analysis comprises of sections based on the objectives of the study. We have three primary objectives and one secondary objective. The primary objectives were: To investigate whether neonatal (cord blood) cytokines and maternal cytokines at delivery are predictive biomarkers of allergic outcomes in children at 18months of age, and also if disease outcome in the child is affected by the pollutants. The secondary objective was to assess the effect of pollutants on immune status of the mother, placenta cytokines, cord blood cytokines and child cytokines. First, we shall focus on multiple regression analysis and later multivariate regression analysis will be tackled. Multiple regression analysis is based on a single imputation and multivariate regression analysis is conducted on multiple imputations.

5.2.1 Analyzing each allergy separately

In this section, associations between each allergy outcome and explanatory variables for primary objectives are investigated using multiple logistic regression. The allergy outcomes include Atopiceczema, Other dermatitis (Otherdet), specific IgE for inhalant allergies in child blood (PhadCHinhalant) and specific IgE for food allergies in child blood (fx5Chfood). Explanatory variables investigated include cord blood cytokines, maternal cytokines and pollutants.

First, associations between cord blood cytokines and each allergy outcome are presented, then associations between maternal cytokines and each allergy outcome and finally we present associations between each allergy outcome and pollutants.

5.2.1.1 Associations between cord blood cytokines and allergy outcomes

Cord blood cytokines examined are il4phacord, il5phacord, il10phacord, il12phacord and INF-gphacord and the allergic outcomes include Atopiceczema, Other dermatitis (Otherdet), specific IgE for inhalant allergies in child blood (PhadCHinhalant) and specific IgE for food allergies in child blood (fx5Chfood). Table 18 contains results of associations between cord blood cytokines and allergy outcomes, obtained by applying model building procedure as described in Section 4.2.3 and Figure 4 presents results obtained by applying prediction methods explained in Section 4.3.

Table 18: Associations between cord blood cytokines and allergic outcomes

Response	Parameter	Estimate (s.e)	P-value	OR (95% C.I.)
Atopic eczema	Intercept	-1.974(0.254)	<.001	-
	il4phacord	0.058(0.020)	0.004	1.060 (1.019,1.102)
	il12phacord	-1.42E-3(6.48E-4)	0.029	0.999 (0.997,1.000)
	IFNgphacord	7.5E-4(3.06E-4)	0.014	1.001 (1.000,1.001)
	Slovakia vs. Belgium	-0.348(0.180)	0.053	0.498 (0.246,1.009)
Otherdet	Intercept	-3.466 (0.269)	<.001	-
	il10phacord	0.001 (0.0004)	0.006	1.001 (1.0002, 1.002)
PhadCHinhalant	Intercept	-6.616 (1.130)	<.001	-
	il5phacord	-0.094 (0.027)	<.001	0.911 (0.864, 0.960)
	IFNgphacord	0.004 (0.001)	0.007	1.004 (1.001, 1.007)
fx5CHfood	Intercept	-2.923 (0.319)	<.001	-
	il10phacord	2.43E-3 (8.36E-4)	0.004	1.002 (1.001,1.004)
	IFNgphacord	1.67E-3 (7.12E-4)	0.019	0.998 (1.0003,1.003)

In Table 18, it is observed that elevated levels of il4phacord and IFNgphacord increase the probability to have Atopic eczema, whereas elevated levels of il12phacord reduce the probability of having Atopic eczema at 18 months of age. The odds of Atopic eczema vs. no Atopic eczema in Slovakia are about half (0.498) the odds of Atopic eczema vs. no Atopic eczema in Belgium. This effect is at borderline and care should be taken in its interpretation.

Increased levels of il10phacord increase the probability to have Otherdermatitis in child at 18months of age. Elevated levels of INFgphacord increase the probability to have inhalant allergies (PhadCHinhalant), whereas elevated levels of il5phacord reduce the probability of having PhadCHinhalant at 18 months of age. Elevated levels of il10phacord and INFgphacord increase the probability to have (food allergies) fx5CHfood at 18 months of age. Hosmer and Lemeshow P-values are 0.108, 0.136, 1.000 and 0.296 for Atopic eczema, Otherdet, PhadCHinhalant and fx5CHfood respectively.

Figure 4 presents ROC curves for associations between cord blood cytokines and allergic outcomes. Cytokines il5phacord and INFgphacord accurately predict PhadCHinhalant (AUC-ROC = 0.987). Variables il4phacord, il12phacord and country provide accurately predict Atopic eczema (0.654). Variables in the final models do not accurately predict Otherdermatitis (AUC-ROC = 0.549) and food allergies (AUC-ROC = 0.138). Confidence intervals and standard deviations for areas under curves are in Table C1.1 in Appendix C.

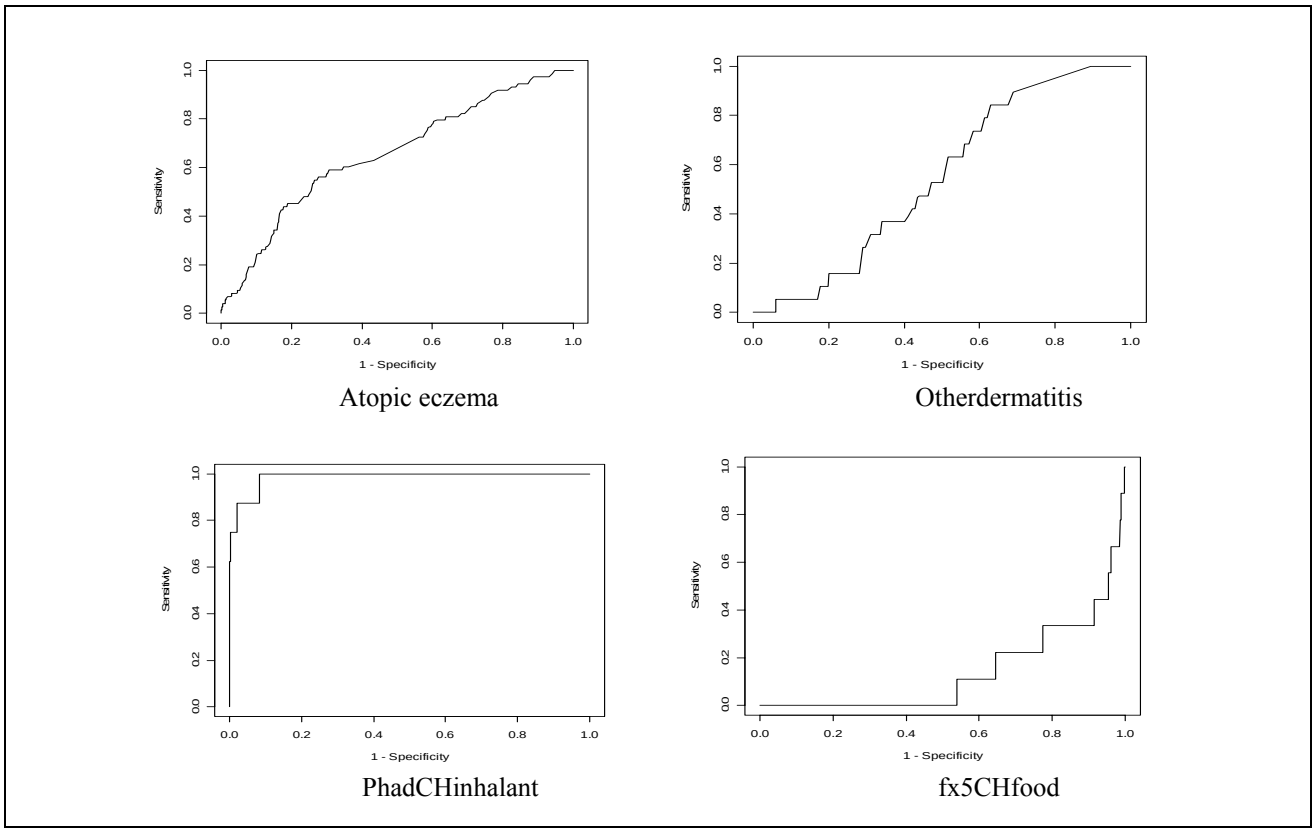

Figure 4: Associations between cord blood cytokines and allergic outcomes

5.2.1.2 Associations between maternal cytokines and allergy outcomes

In this section we assess associations between maternal cytokines and allergy outcomes. Maternal cytokines studied are il4phamaternal, il5phamaternal, il10phamaternal, il12phamaternal and INFgphamaternal and the allergic outcomes comprise of Atopiceczema, Other dermatitis (Otherdet), specific IgE for inhalant allergies in child blood (PhadCHinhalant) and specific IgE for food allergies in child blood (fx5Chfood). Table 19 provides results of the association between maternal cytokines and allergic measures.

Table 19: Association between maternal cytokines and allergic outcomes

Response	Parameter	Estimate (s.e)	P-value	OR (95% C.I.)
Atopic eczema	Intercept	-1.620 (0.146)	<.001	-
	il10phamaternal	-0.002 (0.0005)	0.002	0.998 (0.997, 0.999)
	INFgphamaternal	0.00002 (5.166E-6)	<.001	1.00002 (1.00001, 1.00002)
	Slovakia vs. Belgium	-0.344 (0.177)	0.052	0.362 (0.192, 0.684)
Otherdet	Intercept	-4.023 (0.364)	<.001	-
	il5phamaternal	0.004 (0.002)	0.031	1.004 (1.00008,1.007)
	INFgphamaternal	-0.00003 (9.238E-6)	<.001	0.99997 (0.99995,0.99999)
	il10phamaternal	0.003 (0.001)	0.002	1.003 (1.001,1.005)
	il12phamaternal	0.002 (0.001)	0.001	1.002 (1.001,1.004)
PhadCHinhalant	Intercept	-8.559 (1.990)	<.001	-
	il4phamaternal	0.046 (0.013)	<.001	1.047 (1.021,1.074)
	il12phamaternal	-0.007 (0.002)	<.001	0.993 (0.990,0.997)
fx5CHfood	Intercept	-3.394 (0.276)	<.001	-
	INFgphamaternal	0.00002 (0.00001)	0.042	1.000002 (1.0000004,1.00004)
	il12phamaternal	-0.002 (0.001)	0.039	0.998 (0.997, 0.9999)

Table 19 shows that elevated levels of INFgphamaternal increase the probability to have Atopic eczema, whereas elevated levels of il10phamaternal reduce the probability of having Atopic eczema at 18 months of age. The odds of Atopic eczema vs. no Atopic eczema in Slovakia are about 0.36 the odds of Atopic eczema vs. no Atopic eczema in Belgium.

Increased levels of il5phamaternal, il10phamaternal and il12phamaternal increase the probability to have Otherdermatitis in child at 18months of age whereas elevated levels of INFgphamaternal reduce the probability to have Otherdermatitis. Elevated levels of il4phamaternal increase the probability to have inhalant allergies (PhadCHinhalant), whereas elevated levels of il12phamaternal reduce the probability of having PhadCHinhalant at 18 months of age. Elevated levels of INFgphamaternal increase the probability to have (food allergies) fx5CHfood while increased levels of il12phamaternal reduce the probability to have food allergies at 18 months of age. Hosmer and Lemeshow P-values are 0.454, 0.805, 0.999 and 0.843 for Atopic eczema, Otherdet, PhadCHinhalant and fx5CHfood respectively.

Figure 5 presents ROC curves for associations between maternal cytokines and allergic outcomes. Cytokines il10phamaternal and INFgphamaternal and, country accurately predict Atopic eczema (AUC-ROC=0.656).Cytokines il5phamaternal, il10phamaternal, il12phamaternal and INFgphamaternal accurately predict Otherdermatitis (AUC-ROC=0.801). Cytokines il4phamaternal and il12phamaternal accurately predict PhadCHinhalant (AUC-ROC= 0.979) in child at 18months of age. Maternal cytokines in the final model of fx5CHfood do not predict its occurrence (AUC-ROC = 0.584). Confidence intervals and standard deviations of areas under ROC curves are given in Table C1.2 in Appendix C.

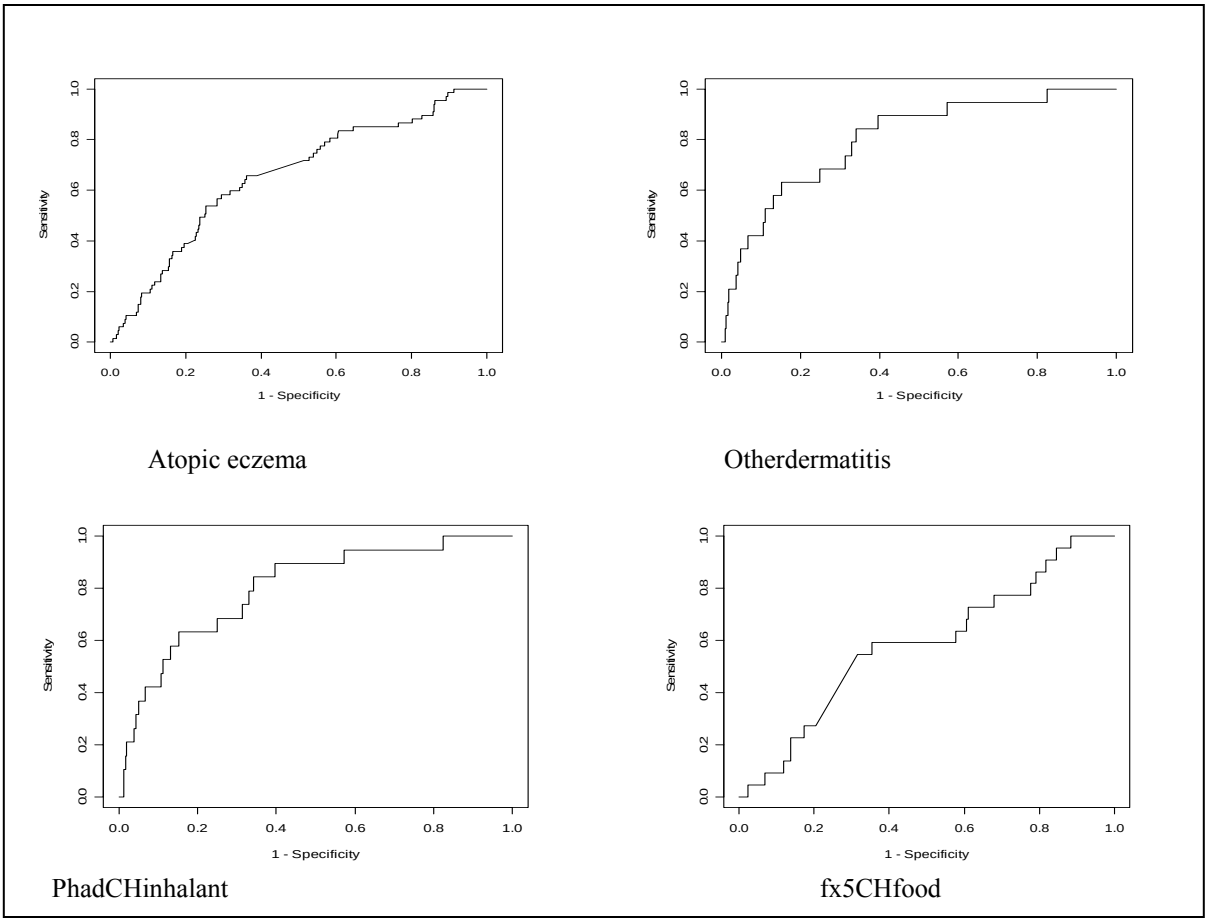

Figure 5: Associations between maternal cytokines and allergic outcomes

5.2.1.3 Associations between pollutants and allergy outcomes

Associations between pollutants and each allergy outcome are studied in this section. Allergy outcomes studied include Atopiceczema, Other dermatitis (Otherdet), specific IgE for inhalant allergies in child blood (PhadCHinhalant) and specific IgE for food allergies in child blood (fx5Chfood). Pollutants studied consist of Lead in maternal blood (Pbblood), Cadmium in maternal blood (Cdblood), Lead in placenta (Pbplacenta), Cadmium in placenta (Cdplacenta), Sum of chlorinated benzenes in maternal blood serum *(MBS-sum1)*, sum of Polychlorinated biphenyls (PCBs) in maternal blood serum *(MBS-sum2)*, sum of organochlorine insecticides in maternal blood serum*(MBS-sum3)* ,sum of chlorinated benzenes in placenta*(PLAT-sum1)* , sum of Polychlorinated biphenyls (PCBs) in placenta*(PLAT-sum2)* , sum of organochlorine insecticides in placenta *(PLAT-sum3)*, sum of chlorinated benzenes in breast milk *(BM-sum1)*, sum of Polychlorinated biphenyls (PCBs) in breast milk*(BM-sum2)* , and sum of organochlorine insecticides in breast milk *(BM-sum3)*. Results of associations between pollutants and allergy outcomes are given in Table 20.

Table 20: Associations between pollutants and allergic outcomes

Response	Parameter	Estimate (s.e)	P-value	OR (95% C.I.)
Atopic eczema	Intercept	-2.128 (0.184)	<.001	-
	PLAT-sum3	-0.335 (0.097)	<.001	0.716 (0.592,0.865)
	BM-sum1	-0.105 (0.029)	<.001	0.900 (0.849,0.954)
	BM-sum3	0.224 (0.044)	<.001	1.251 (1.148,1.363)
Otherdet	Intercept	-3.477 (0.272)	<.001	-
	Cdblood	0.203 (0.101)	0.044	1.225 (1.005,1.492)
	BM-sum1	-0.139 (0.051)	0.007	0.871 (0.788,0.962)
	BM-sum3	0.138 (0.058)	0.018	1.147 (1.024,1.286)
PhadCHinhalant	Intercept	-7.983 (1.774)	<.001	-
	PLAT-sum3	0.151 (0.063)	0.017	1.163 (1.028,1.316)
	BM-sum1	0.219 (0.083)	0.008	1.245 (1.058,1.464)
	BM-sum2	-0.339 (0.149)	0.023	0.712 (0.532,0.954)
	Pbplacenta	13.959 (4.002)	<.001	>999.999 (453.398, >999.999)
fx5CHfood	Intercept	-3.652 (0.342)	<.001	-
	MBS-sum1	0.104 (0.031)	<.001	1.110 (1.045,1.178)
	MBS-sum2	-0.128 (0.063)	0.043	0.880 (0.777,0.996)

Table 20 reveals that, increased concentrations of the sum of organochlorine insecticides in placenta (PLAT-sum3) and sum of Chlorinated benzenes in breast milk (BM-sum1) reduced the probability of Atopic eczema while increased sum of organochlorine insecticides in breast milk (BM-sum3) increased chances of Atopiceczema.

Increased concentrations of Cadmium in maternal blood serum (Cdblood) and sum of organochlorine insecticides in breast milk (BM-sum3) increase the probability of Otherdermatitis whereas increased levels of the sum of Chlorinated benzenes in breast milk (BM-sum1) reduced the probability of Otherdermatitis.

Higher levels of the sum of organochlorine insecticides in placenta (PLAT-sum3), sum of Chlorinated benzenes in breast milk (BM-sum1) and Lead in placenta (Pbplacenta) increased the probability of specific IgE for inhalant allergies in child blood (PhadCHinhalant) whereas, higher levels of the sum of PCBs in breast milk (BM-sum2) reduced the probability of PhadCHinhalant.

Increased concentration of the sum of Chlorinated benzenes in maternal blood serum (MBS-sum1) increased chances of food allergies in child blood (fx5Chfood) and increased concentrations of the sum of PCBs in maternal blood (MBS-sum2) reduce chances of fx5Chfood. Hosmer and Lemeshow P-values are 0.293, 0.946 = 0.895 and 0.095 for Atopic eczema, Otherdet, PhadCHinhalant and fx5CHfood respectively.

Figure 6 presents ROC curves for associations between pollutants and allergic outcomes. Sum of Organochlorine insecticides in placenta (PLAT-sum3), sum of Chlorinated benzenes in breast milk (BM-sum1) and sum of Organochlorine insecticides in breast milk (BM-sum3) accurately predict Atopic eczema (AUC-ROC =0.697), Lead in placenta (Pbplacenta), sum of Organochlorine insecticides in placenta (PLAT-sum3), sum of Chlorinated benzenes in breast milk (BM-sum1) and sum of PCBs in breast milk (BM-Sum2) accurately predict PhadCHinhalant (AUC-ROC = 0.972) and sum of Chlorinated benzenes in maternal blood serum (MBS-sum1), sum of PCBs in maternal blood serum (MBS-sum2) accurately predict fx5CHfood (AUC-ROC = 0.756) in child at 18 months of age. Pollutants in the final model of Otherdermatitis do not predict it occurrence although they

affect it. Confidence intervals and standard deviations of areas under ROC curves are given in Table C1.3 in Appendix C.

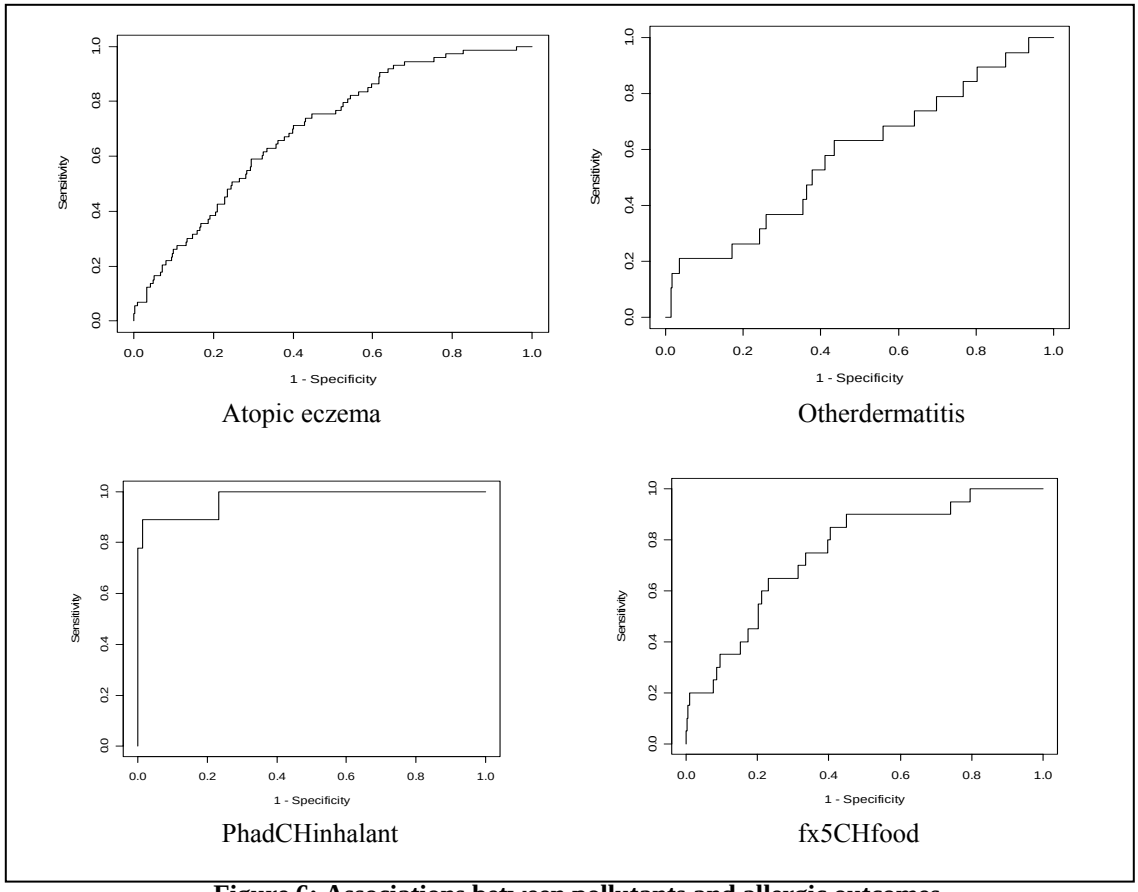

Figure 6: Associations between pollutants and allergic outcomes

5.2.2 Associations between cumulative allergies and explanatory variables

Associations between cumulative allergies and explanatory variables are presented in this section. The cumulative allergy was 1 for a subject with atleast one of the four allergies and 0 for a subject with none of the four allergies (Atopiceczema, Other dermatitis (Otherdet), specific IgE for inhalant allergies in child blood (PhadCHinhalant) and specific IgE for food allergies in child blood (fx5Chfood)). Explanatory variables studied include cord blood cytokines, maternal cytokines and pollutants.

5.2.2.1 Associations between cord blood cytokines and cumulated allergies

In this section we present associations between cord blood cytokines and cumulated allergies. Table 21 below shows that elevated levels of il4phacord in the cord blood of the mother increases the probability of atleast one of the four allergies in child at 18 months of age (Hosmer and Lemeshow P-value = 0.252).

Table 21: Associations between cord blood cytokines and cumulated allergies

Parameter	Estimate (s.e)	P-value	OR (95 C.I.)
Intercept	-1.465 (0.169)	<.0001	-
il4phacord	0.0445 (0.0143)	0.002	1.046 (1.017, 1.075)

Cytokine il4phacord accurately predicts atleast one of the four allergies (AUC-ROC =0.616) with confidence interval of (0.551, 0.681) and standard deviation of 0.032. Figure 7 presents the ROC curve for the associations between cord blood cytokines and cumulated allergies.

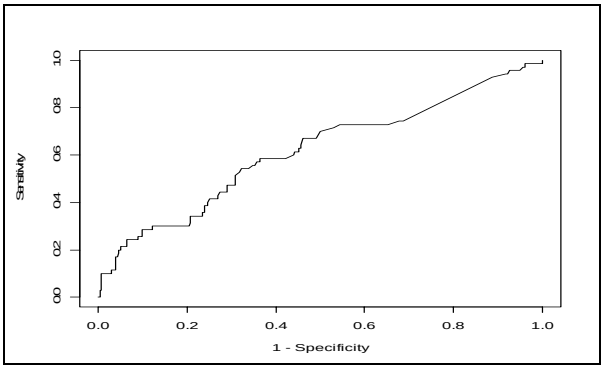

Figure 7: Associations between cord blood cytokines and cumulated allergies.

5.2.2.2 Associations between maternal cytokines and cumulated allergies

Associations between maternal cytokines and cumulated allergies are presented in this section. As we can observe in Table 22 below, the odds of atleast one allergy vs. no allergy in Stara Lubovna is about 45% the odds of allergy vs. no allergy in Mol. Hosmer and Lemeshow P-value for this model is 1.000.

Table 22: Associations between maternal cytokines and cumulated allergies

Parameter	Estimate (s.e)	P-value	OR (95 C.I.)
Intercept	-1.251 (0.144)	<.001	-
Bratislava vs. Mol	0.497 (0.190)	0.009	1.412 (0.761, 2.618)
Stara Lubovna vs. Mol	-0.649 (0.224)	0.004	0.448 (0.214, 0.938)

The variable region accurately predicts the probability of having atleast one of the four allergies (AUC-ROC = 0.618) with confidence interval of (0.553, 0.683) and standard deviation of 0.032. The ROC curve for the associations between maternal cytokines and cumulated allergies is presented in Figure 8 below.

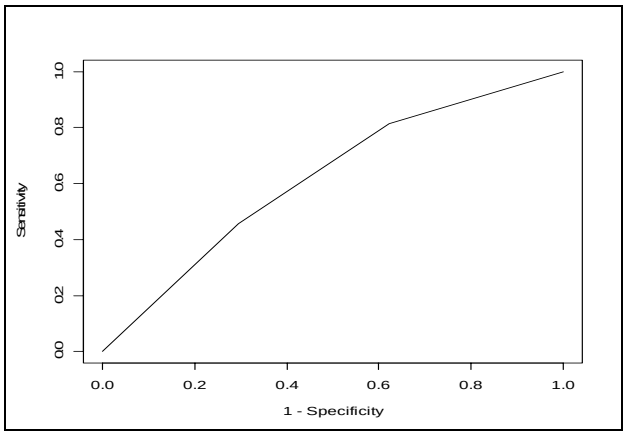

Figure 8: associations between maternal cytokines and cumulated allergies

5.2.2.3 Associations between pollutants and cumulated allergies

This section presents associations between pollutants and cumulated allergies. Table 23 below shows that increased levels of BM-sum3 and Pbplacenta increase the probability of having atleast one of the four allergies whereas, increased PLAT-sum3 and Pbblood reduce the probability of having atleast one allergy in child at 18 months of age.

Table 23: Associations between pollutants and cumulated allergies

Parameter	Estimate (s.e)	P-value	OR (95 C.I.)
Intercept	-1.029 (0.233)	<.001	-
PLAT-sum3	-0.079 (0.044)	0.072	0.923 (0.846, 1.007)
BM-sum3	0.107 (0.032)	<.001	1.113 (1.047, 1.184)
Pbblood	-0.016 (0.007)	0.024	0.984 (0.970, 0.998)
Pbplacenta	4.562 (1.691)	0.007	95.764 (3.485, >999.999)

The variables in the final model accurately predict the probability of having atleast one allergy (AUC-ROC = 0.774), with confidence interval of (0.717, 0.832) and standard deviation of 0.029. Figure 9 below shows the ROC curve of the preceding model.

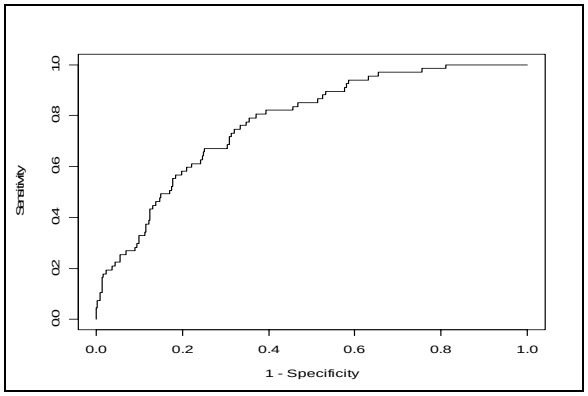

Figure 9: Associations between pollutants and cumulated allergies

5.2.2.4 Summary from multiple logistic regression analysis

From the associations between cord blood cytokines and allergy outcomes, variables il4phacord, il12phacord, INFgphacord and country were associated with Atopic eczema. Cytokine il10phacord affected Otherdermatitis and food allergies, and il5phacord and INFgphacord affected inhalant allergies. Except for Otherdermatitis and food allergies, variables associated with Atopic eczema and inhalant allergies accurately predicted their occurrence.

For the associations between maternal cytokines and allergy outcomes, variables il10phamaternal and INFgphamaternal affected Atopic eczema. Cytokines il5phamaternal, il10phamaternal, il12phamaternal and INFgphamaternal were associated with Otherdermatitis. Cytokines il4phamaternal and il12phamaternal affected inhalant allergies and, INFgphamaternal and il12phamaternal were associated with food allergies. Apart from food allergies, variables associated with Atopic eczema, Otherdermatitis and inhalant allergies accurately predicted them.

Variables PLAT-sum3, BM-sum1, BM-sum3 were associated with Atopic eczema. Cdblood, BM-sum1 and BM-sum3 affected Otherdermatitis. Inhalant allergies were affected by PLAT-sum3, BM-sum1, BM-sum2 and Pbplacenta. Food allergies were affected by MBS-sum1 and MBS-sum2. All allergies were accurately predicted by variables associated with them except Otherdermatitis.

From the cumulated response, il4phacord, region, PLAT-sum3, BM-sum3, Pbblood and Pbplacenta affected probability of any of the allergies. All these variables accurately predicted probability of any of the allergies.

All these variables were used to fit multivariate logistic regression models for primary objectives and the Wald test was used for model reduction. For the secondary objective only multiple regression analysis was done. Therefore, results presented for multivariate logistic regression analysis are for primary objectives only.

5.2.3 Relationships between pollutants and immune status of the mother, placenta cytokines, cord blood cytokines and child cytokines

The secondary objective is answered in this section using multiple regression analysis. Relationships between pollutants and immune status of the mother, placenta cytokines, cord blood cytokines and child cytokines are explored. Pollutants were measured in maternal blood, placenta and breast milk. They include Lead in maternal blood (Pbblood), Cadmium in maternal blood (Cdblood), Lead in placenta (Pbplacenta), Cadmium in placenta (Cdplacenta), Sum of chlorinated benzenes in maternal blood serum *(MBS-sum1)*, sum of Polychlorinated biphenyls (PCBs) in maternal blood serum *(MBS-sum2)*, sum of organochlorine insecticides in maternal blood serum*(MBS-sum3)* ,sum of chlorinated benzenes in placenta*(PLAT-sum1)* , sum of Polychlorinated biphenyls (PCBs) in placenta*(PLAT-sum2)* , sum of organochlorine insecticides in placenta *(PLAT-sum3)*, sum of chlorinated benzenes in breast milk *(BM-sum1)*, sum of Polychlorinated biphenyls (PCBs) in breast milk*(BM-sum2)* , and sum of organochlorine insecticides in breast milk *(BM-sum3)*.

The immune status of the mother was measured by her allergic history, atopic status and TotalIgE. Placenta cytokines considered are il4placenta, il5placenta, il10placenta, tnfplacenta and

INFgplacenta, cord blood cytokines include il4phacord, il5phacord, il10phacord, il12phacord and INFgphacord and child cytokines are il2child, il4child, il5child, il10child, and INFgchild.

Except allergic history and atopic status of the mother which are binary outcomes, TotalIgE, placenta cytokines, cord blood cytokines and child cytokines are continuous. Thus, Multiple Logistic regression analysis was done to capture associations between pollutants and, allergic history and atopic status of the mother. Multiple regression analysis was used to obtain relationships between pollutants and TotalIgE, pollutants and placenta cytokines, pollutants and cord blood cytokines and pollutants and child cytokines.

Results obtained from both regression procedures are presented in this order: First associations between pollutants and, allergic history, atopic status and TotalIgE of the mother are presented, relationships between placenta cytokines and pollutants, cord blood cytokines and pollutants and we conclude with child cytokines and pollutants in.

5.2.3.1 Association between pollutants and Allergic history of the mother

Elevated levels of Lead in maternal blood serum (Pbblood) and sum of Organochlorine insecticides in breast milk (BM-sum3) reduce the probability of a mother being allergic in the past. The Hosmer and Lemeshow P-value is 0.461 (Table C2.1 in Appendix C).

5.2.3.2 Association between pollutants and Atopic status of the mother

The doubtful level of Atopic status was combined with mothers who were not atopic. Higher levels of the sum of PCBs in maternal blood serum (MBS-sum2) and sum of Organochlorine insecticides in breast milk (BM-sum3) reduce the probability of Atopy among mothers. Increased levels of the sum of PCBs in breast milk increase probability of Atopy among mothers. The Hosmer and Lemeshow P-value is 0.803 (Table C2.1 in Appendix C).

5.2.3.3 Relationship between pollutants and TotalIgE of the mother

The sum of Chlorinated benzenes in placenta (PLAT-sum1) and sum of PCBs in breast milk (BM-sum2) increase TotalIgE whereas sum of PCBs in placenta (PLAT-sum2) and sum of Organochlorine insecticides in breast milk (BM-sum3) reduce TotalIgE (Table C2.1 in Appendix C).

5.2.3.4 Relationships between pollutants and placenta cytokines

Concentrations of Lead in maternal blood serum (Pbblood), sum of Organochlorine insecticides in maternal blood serum (MBS-sum3), sum of PCBs in placenta (PLAT-sum2) and sum of Organochlorine insecticides in breast milk (BM-sum3) reduce levels of cytokine INFgplacenta. Concentrations of Lead in placenta (Pbplacenta), sum of PCBs in maternal blood serum (MBS-sum2), sum of Chlorinated benzenes in placenta (PLAT-sum1) and sum of Organochlorine insecticides in placenta (PLAT-sum3) elevate levels INFgplacenta (Table C2.2 in Appendix C).

Lead in maternal blood serum (Pbblood), sum of PCBs in placenta (PLAT-sum2), sum of Chlorinated benzenes in breast milk (BM-sum1), sum of Organochlorine insecticides in breast milk (BM-sum3) il4placenta cytokines. Lead in placenta (Pbplacenta), sum of Chlorinated benzenes in placenta (PLAT-sum1), sum of Organochlorine insecticides in placenta (PLAT-sum3) and sum of PCBs in breast milk (BM-sum2) increase il4placenta cytokines (Table C2.3 in Appendix C).

Lead in maternal blood serum (Pbblood), sum of PCBs in placenta (PLAT-sum2), sum of Chlorinated benzenes in breast milk (BM-sum1) and sum of Organochlorine insecticides in breast milk (BM-sum3) decrease il5placenta cytokines Lead in placenta (Pbplacenta), sum of PCBs in maternal blood serum (MBS-sum2), sum of Chlorinated benzenes in placenta (PLAT-sum1), sum of Organochlorine insecticides in placenta (PLAT-sum3) and sum of PCBs in breast milk (BM-sum2) elevates levels of il5placenta cytokines (Table C2.4 in Appendix C).

Lead in maternal blood (Pbblood), sum of PCBs in placenta (PLAT-sum2), sum of Organochlorine insecticides in placenta (PLAT-sum3), sum of Chlorinated benzenes in breast milk (BM-sum1), and sum of PCBs in breast milk (BM-sum2) reduces levels of il10placenta cytokine. Cadmium in placenta (Cdplacenta), sum of PCBs in maternal blood serum (MBS-sum2) and sum of Chlorinated benzenes in placenta (PLAT-sum1) increase levels of il10placenta cytokine (Table C2.5 in Appendix C).

Lead in placenta (Pbplacenta), Cadmium in placenta (Cdplacenta), sum of Chlorinated benzenes in maternal blood (MBS-sum1), sum of Chlorinated benzenes in placenta (PLAT-sum1), sum of Organochlorine insecticides in placenta (PLAT-sum3) and sum of Chlorinated benzenes in breast milk (BM-sum1) increase levels of tnfplacenta cytokines. Sum of PCBs in the placenta (PLAT-sum2) and sum of Organochlorine insecticides in breast milk (BM-sum3) decrease levels of tnfplacenta cytokines (Table C2.6 in Appendix C)

5.2.3.5 Relationships between pollutants on cord blood cytokines

Lead in maternal blood serum (Pbblood), sum of Chlorinated benzenes in breast milk (BM-sum1) and sum of PCBs in breast milk (BM-sum2) decrease levels of INFgphacord cytokines. Cadmium in maternal blood serum (Cdblood), sum of PCBs in maternal blood serum (MBS-sum2) and sum of Organochlorine insecticides in breast milk (BM-sum3) increase levels of INFgphacord cytokines (Table C2.7 in Appendix C)

Lead in maternal blood serum (Pbblood), sum of Chlorinated benzenes in maternal blood serum (MBS-sum1) and sum of PCBs in breast milk (BM-sum2) decrease levels of il4phacord cytokines. Lead in placenta (Pbplacenta) and Cadmium in maternal blood serum (Cdblood) increase levels of il4phacord cytokines (Table C2.8 in Appendix C).

Lead in maternal blood serum (Pbblood), Lead in placenta (Pbplacenta), sum of organochlorine insecticides in maternal blood serum (MBS-sum3), PLAT-sum3, BM-sum1 and BM-sum2 reduce levels of il5phacord cytokines. Cadmium in maternal blood (Cdblood), sum of PCBs in maternal blood serum (MBS-sum2) and sum of organochlorine insecticides in breast milk (BM-sum3) elevate levels of il5phacord cytokines (Table C2.9 in Appendix C).

Lead in maternal blood serum (Pbblood), sum of PCBs in maternal blood serum (MBS-sum2), sum of Chlorinated benzenes in breast milk (BM-sum1) and sum of Organochlorine insecticides in breast milk (BM-sum3) reduce levels of il10phacord cytokines. Lead in placenta (Pbplacenta), Cadmium in maternal blood (Cdblood), the sum of Organochlorine insecticides in maternal blood (MBS-sum3) and the sum of Organochlorine insecticides in placenta (PLAT-sum3) increase levels of il10phacord cytokines (Table C2.10 in Appendix C).

Lead in maternal blood serum (Pbblood), the sum of Organochlorine insecticides in placenta (PLAT-sum3) and sum of PCBs in breast milk (BM-sum2) reduce levels of il12phacord cytokines.

Lead in placenta (Pbplacenta), Cadmium in maternal blood (Cdblood), sum of PCBs in placenta (PLAT-sum2) and sum of Chlorinated benzenes in breast milk (BM-sum1) increase levels of il12phacord cytokines (Table C2.11 in Appendix C).

5.2.3.6 Relationships between pollutants on Child cytokines

Lead in maternal blood serum (Pbblood) and sum of PCBs in maternal blood serum (MBS-sum2) reduce levels of INFgchild cytokines. Lead in placenta (Pbplacenta), Cadmium in blood (Cdblood), sum of Chlorinated benzenes in maternal blood serum (MBS-sum1), sum of Organochlorine insecticides in maternal blood serum (MBS-sum3) and sum of Chlorinated benzenes in placenta (PLAT-sum1) increase levels of INFgchild cytokines (Table C2.12 in Appendix C).

Cadmium in maternal blood serum (Cdblood) and the sum PCBs in breast milk (BM-sum2) increase in the levels of il4child cytokines. Pbblood, Pbplacenta, Cdplacenta and PLAT-sum2 reduce levels of il4child cytokines (Table C2.13 in Appendix C).

Lead in maternal blood (Pbblood), sum of PCBs in maternal blood serum (MBS-sum2), sum of Chlorinated benzenes in placenta (PLAT-sum1) decrease levels of il5child cytokines. Lead in placenta (Pbplacenta), Cadmium in maternal blood serum (Cdblood), Cadmium in placenta (Cdplacenta) and sum of Chlorinated benzenes in breast milk (BM-sum1) increase levels of il5child cytokines (Table C2.14 in Appendix C)

Lead in maternal blood (Pbblood), Lead in placenta (Pbplacenta), Cadmium in maternal blood serum (Cdblood), sum of PCBs in maternal blood serum (MBS-sum2) decrease levels of il10child cytokines. Sum of Chlorinated benzenes in maternal blood serum (MBS-sum1), sum of Organochlorine insecticides in maternal blood serum (MBS-sum3) and sum of Organochlorine insecticides in placenta (PLAT-sum3) elevate levels of il10child cytokines (Table C2.15 in Appendix C).

Lead in placenta (Pbplacenta), Cadmium in placenta (Cdplacenta), sum of PCBs in maternal blood serum (MBS-sum2) and sum of PCBs in placenta (PLAT-sum2) reduce levels of il2child cytokines. Cadmium in blood (Cdblood), sum of Chlorinated benzenes in maternal blood serum (MBS-sum1), sum of Chlorinated benzenes in placenta (PLAT-sum1) and sum of PCBs in breast milk (BM-sum2) increase levels of il2child cytokines (Table C2.16 in Appendix C).

5.2.4 Multivariate logistic regression

The selection of final models for the primary objectives of the study is described in this section. First multivariate logistic regression models were fitted on a data set with complete cases and model reduction was done using the Wald test. Multiple imputations were then done on the original data set and multivariate logistic regression models were fitted on the imputed data set. Further, the allergy outcome of specific IgE for inhalant allergies in child blood (PhadCHinhalant) had only 3 events. For this reason this response was dropped and multivariate logistic models were refitted with three responses (Atopic eczema, Otherdet and Fx5CHfood). Results of final models on complete case data set, imputed data sets and a data set without PhadCHinhalant under various objectives are provided in the subsequent sections. Also, ROC curves, areas under these curves and their confidence intervals are presented. ROC curves were only done on imputed data set.

5.2.4.1 Associations between cord blood cytokines and allergy outcomes

All cord blood cytokines were used as explanatory variables to fit multivariate logistic regression models and models were reduced using the Wald test. Cord blood cytokines included il4phacord, il5phacord, il10phacord, il12phacored and INFgphacord. Allergy outcomes included Atopiceczema, Other dermatitis (Otherdet), specific IgE for inhalant allergies in child blood (PhadCHinhalant) and specific IgE for food allergies in child blood (fx5Chfood).

Results for the final model on complete case data set from associations between cord blood cytokines and allergy outcomes are given in Table 24. Elevated levels of il4phacord cytokine in the cord blood of the mother increase the probability of manifesting all four allergies while taking into account the association between them.

Table 24: Results from complete cases data

Parameter	Estimate (s.e)	95% (C.I.)
Intercept	-2.812(0.162)	(-3.130,-2.494)
il4phacord	0.037(0.013)	(0.011,0.063)

Results from imputed data sets with and without PhadCHinhalant outcome are given in Table 25. Models with and without PhadCHinhalant are different in a way that they have different explanatory variables. In a model with PhadCHinhalant, elevated levels of il5phacord and il10phacord increase the probability of having the four allergies while taking into account the association between them. This model has more explanatory variables than a model fitted on complete cases data. In a model without PhadCHinhalant, elevated levels of il4phacord increase the probability of manifesting all four allergies. Results of this model are similar to results from a model fitted on complete cases data set.

Table 25: Results from imputed data sets

	With PhadCHinhalant		Without PhadCHinhalant	
Parameter	Estimate (s.e)	95% (C.I.)	Estimate (s.e)	95% (C.I.)
Intercept	-2.856 (0.1309)	(-3.113, -2.599)	-2.661 (0.137)	(-2.929, -2.392)
il4phacord	-	-	0.039 (0.012)	(0.017, 0.063)
il5phacord	0.0113 (0.0029)	(0.006, 0.017)	-	-
il10phacord	0.0010 (0.0003)	(0.0004, 0.002)	-	-

Figure 10 below presents ROC curves for models with and without PhadCHinhalant (Table 25). Cytokines il4phacord, il5phacord and, il10phacord accurately predict the probability of manifesting Atopic eczema (AUC-ROC = 0.719 and 0.686; with and without PhadCHinhalant respectively). Cytokines in the final model cannot accurately predict Otherdermatitis, PhadCHinhalant and fx5CHfood .Table C3.1 in Appendix C provides more information.

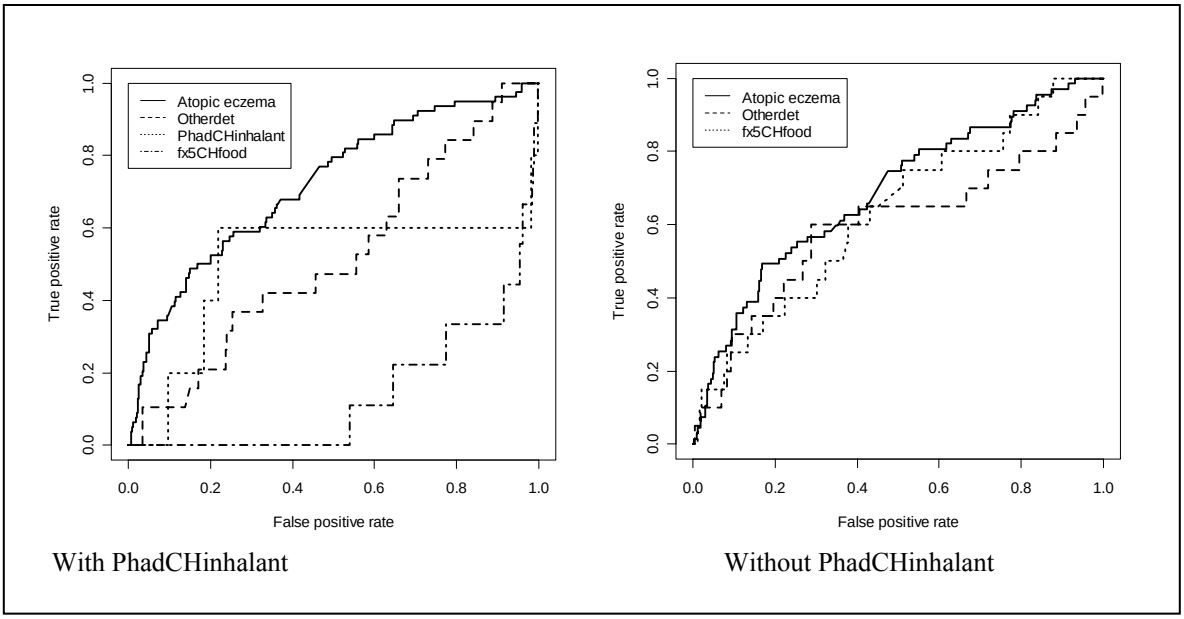

Figure 10: Associations between cord blood cytokines and allergic outcomes

5.2.4.2 Associations between maternal cytokines and allergy outcomes

All maternal blood cytokines (il4phamaternal, il5phamaternal, il10phamaternal, il12phamaternal and INFgphamaternal) were used as explanatory variables to fit multivariate logistic regression models and model reduction was done using the Wald test. Allergy outcomes included Atopiceczema, Other dermatitis (Otherdet), specific IgE for inhalant allergies in child blood (PhadCHinhalant) and specific IgE for food allergies in child blood (fx5Chfood. Results for the final model on the complete cases data are given in Tables 26. Elevated levels of INFgphamaternal in the maternal blood serum increase the probability of developing all four allergies while associations between them are taken into account.

Table 26: Results from complete cases data

Parameter	Estimate (s.e)	95% C.I.
Intercept	-2.778 (0.114)	(-3.001,-2.555)
INFgphamaternal	1.9E-5 (5.166E-6)	(8.8746E-6,2.9125E-6)

Table 27 provides results from imputed data sets. Models from data with and without Phadinhalant are different in a sense that both models have different explanatory variables. Results from data with Phadinhalant disclose that the probability of having allergies is lower in Slovakia compared to Belgium if the associations between allergies are taken into account. From a model without Phadinhalant, increased levels of INFgphamaternal cytokines increase the probability of having allergies when we take associations between allergies into consideration. This result is similar to the result obtained from complete case data.

Table 27: Results from imputed data

	With PhadCHinhalant		Without PhadCHinhalant	
Parameter	Estimate (s.e)	95% (C.I.)	Estimate (s.e)	95% (C.I.)
Intercept	-2.169(0.164)	(-2.490,-1.849)	-2.576(0.135)	(-2.840,-2.312)
INFgphamaternal	-	-	1.9E-5 (5.166E-6)	(8.8746E-6,2.9125E-6)
Slovakia	-0.582(0.216)	(-1.005,-0.159)	-	-
Belgium	0.000(0.000)	(0.000,0.000)	-	-

Figure 11 below presents ROC curves for models in Table 27. Country accurately predicts food allergies (AUC-ROC = 0.672) and Atopic eczema (AUC-ROC = 0.629) in the presence of PhadCHinhalant. Country does not accurately predict Otherdermatitis and PhadCHinhalant. INFgphamaternal accurately predicts Atopic eczema (AUC-ROC=0.611) and Otherdermatitis (AUC-ROC=0.730) in the absence of PhadCHinhalant but does not accurately predict food allergies. Table C3.2 in Appendix C provides more details.

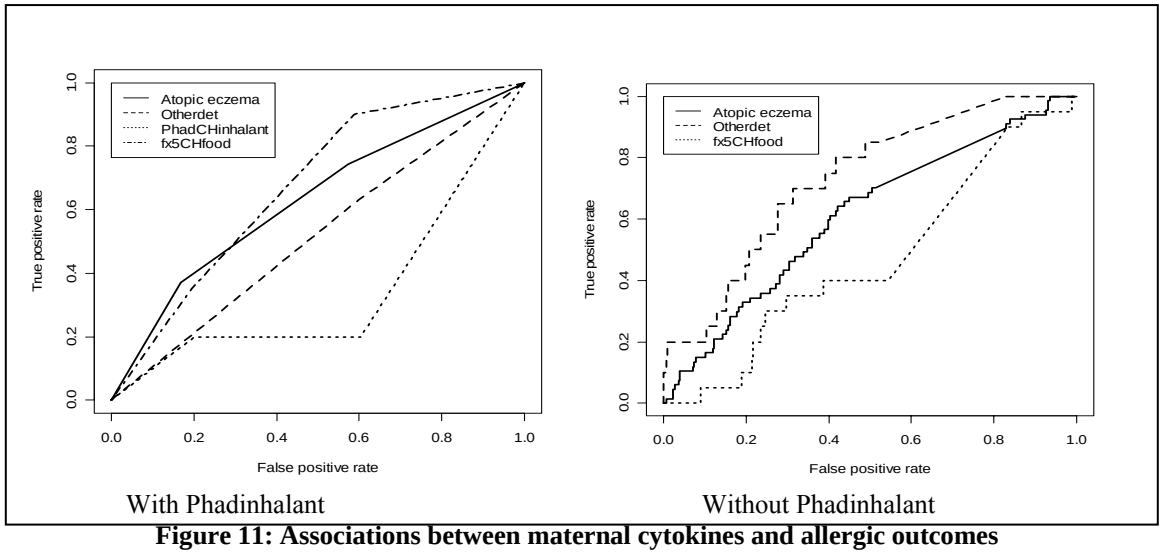

Figure 11: Associations between maternal cytokines and allergic outcomes

5.2.4.3 Associations between pollutants and allergy outcomes

All pollutants were considered as explanatory variables in the multivariate logistic regression model. They include Lead in maternal blood (Pbblood), Cadmium in maternal blood (Cdblood), Lead in placenta (Pbplacenta), Cadmium in placenta (Cdplacenta), Sum of chlorinated benzenes in maternal blood serum *(MBS-sum1)*, sum of Polychlorinated biphenyls (PCBs) in maternal blood serum *(MBS-sum2)*, sum of organochlorine insecticides in maternal blood serum*(MBS-sum3)* ,sum of chlorinated benzenes in placenta*(PLAT-sum1)* , sum of Polychlorinated biphenyls (PCBs) in placenta*(PLAT-sum2)* , sum of organochlorine insecticides in placenta *(PLAT-sum3)*, sum of chlorinated benzenes in breast milk *(BM-sum1)*, sum of Polychlorinated biphenyls (PCBs) in breast milk*(BM-sum2)* , and sum of organochlorine insecticides in breast milk *(BM-sum3)*.Disease outcomes include Atopiceczema, Other dermatitis (Otherdet), specific IgE for inhalant allergies in child blood (PhadCHinhalant) and specific IgE for food allergies in child blood (fx5Chfood).

Results of models fitted on complete case data are presented in Table 28. Increased levels of the sum of organochlorine insecticides in breast milk (BM-sum3) increase the probability of developing

all four allergies whereas increased levels of Lead in maternal blood serum (Pbblood) and sum of Chlorinated benzenes in maternal blood serum (MBS-sum1) reduce the probability of developing all four allergies when associations between allergies is considered.

Table 28: Results from complete cases data

Parameter	Estimate (s.e)	95% (C.I.)
Intercept	-2.148 (0.292)	(-2.721,-1.576)
Pbblood	-0.019 (0.009)	(-0.038,-0.002)
MBS-sum1	-0.142 (0.046)	(-0.233,-0.051)
BM-sum3	0.066 (0.031)	(0.005, 0.128)

Table 29 shows results from imputed data. Increased levels of Lead in maternal blood serum (Pbblood), sum of PCBs in maternal blood serum (MBS-sum2) and sum of PCBs in breast milk (BM-sum2) reduce the probability of manifesting all allergies by accounting for associations between them. On the contrary, increased levels of Cadmium in placenta (Cdplacenta), sum of Organochlorine insecticides in maternal blood (MBS-sum3) and sum of Organochlorine insecticides breast milk (BM-sum3) increase the probability of having all allergies. A model without PhadCHinhalant had fewer explanatory variables. However conclusions from these variables are consistent with those from a model that incorporates PhadCHinhalant. Models fitted on imputed data have more explanatory variables than a model fitted on complete case data.

Table 29: Results from imputed data sets

	With Phadinhalant		Without Phadinhalant	
Parameter	Estimate (s.e)	95% (C.I.)	Estimate (s.e)	95% (C.I.)
Intercept	-2.629 (0.223)	(-3.065,-2.192)	-2.181 (0.199)	(-2.573,-1.789)
Pbblood	-0.013 (0.006)	(-0.025,-0.000)	-0.017 (0.006)	(-0.029,-0.005)
Cdplacenta	27.959 (7.100)	(14.043,41.875)	-	-
MBS-sum2	-0.052 (0.024)	(-0.099,-0.004)	-	-
MBS-sum3	0.059 (0.022)	(0.016,0.101)	-	-
PLAT-sum2	-	-	-0.107 (0.037)	(-0.179,-0.034)
BM-sum2	-0.079 (0.026)	(-0.129,-0.029)	-0.051 (0.024)	(-0.097,-0.004)
BM-sum3	0.058 (0.026)	(0.007,0.108)	0.127 (0.023)	(0.081,0.173)

Figure 12 below presents ROC curves of models in Table 29. The variables in a model with PhadCHinhalant accurately predict Atopic eczema (AUC-ROC = 0.669), Otherdermatitis (AUC-ROC = 0.649) and food allergies (AUC-ROC = 0.739) but they do not predict PhadCHinhalant AUC-ROC = 0.317). Variables in a model without PhadCHinhalant only predict Atopic eczema accurately (AUC-ROC = 0.704). Table C3.3 in Appendix C contains more details.

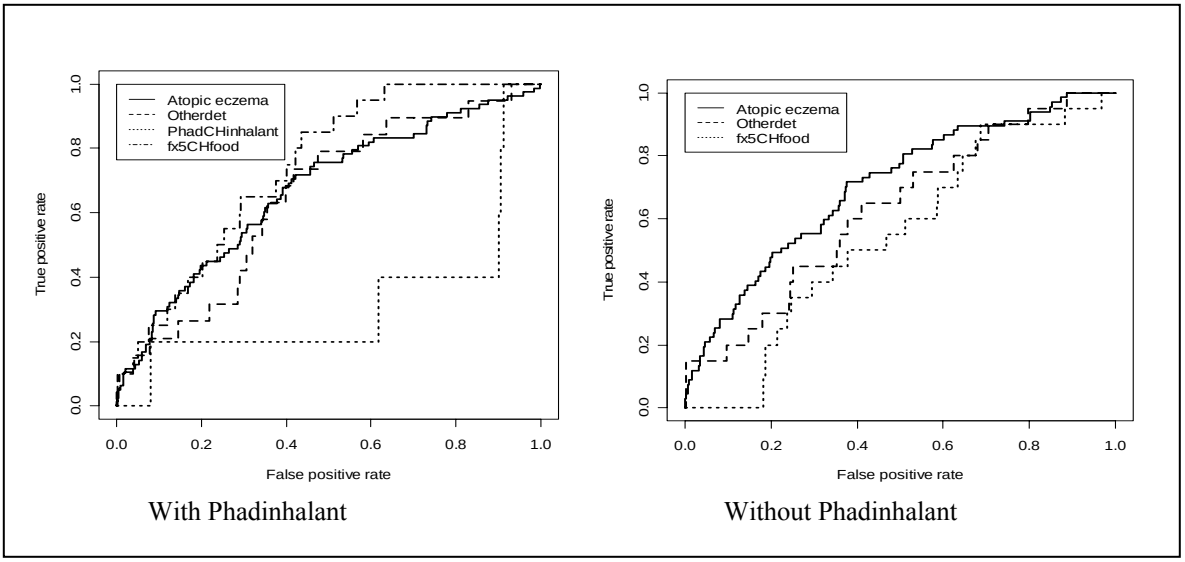

Figure 12: Associations between pollutants and allergic outcomes

5.3 Diagnostics of multivariate logistic models

Diagnostics for the multivariate logistic models were carried out to inspect the adequacy of the models. In this section, plots of Pearson residuals against predicted probabilities using the Lowess method for various models are presented. If the model fits well, a Lowess smooth curve between the two lines of the scatter plot of the Pearson residuals against predicted probabilities is expected to be horizontal with an intercept of zero (Kutner *et al*., 5th edition 2005).

Figure D1 in Appendix D shows plots of Pearson residuals versus predicted probabilities for multivariate logistic models of primary objectives using the Lowess method. The dotted lines show the scatter plot of the Pearson residuals against predicted probabilities. In all cases, the Lowess smooth curve sandwiched between the two dotted lines approximates a line having zero intercept and slope, and we conclude that no significant model inadequacy is apparent.

5 Discussion

The study comprised of 500 mothers and 500 children from three countries and five regions. The countries included Slovakia, Romania and Belgium. Regions of Brastislava and Stara Lubovna were selected from Slovakia, Bucharest and Giurgiu regions from Romania and Mol region from Belgium.

The study was designed to investigate whether neonatal (cord blood) cytokines were predictive biomarkers of allergic outcomes in children at 18months of age, examine if maternal cytokines at delivery were predictive biomarkers of allergic outcomes in children at 18months of age, and also to evaluate if disease outcome in the child is affected by the pollutants. As a secondary aim, the effect of pollutants on immune status of the mother, placenta cytokines, cord blood cytokines and child cytokines was to be assessed.

Allergic outcomes investigated in this study were Atopiceczema, Other dermatitis (Otherdet), specific IgE for inhalant allergies in child blood (PhadCHinhalant) and specific IgE for food allergies in child blood (fx5Chfood) at 18 months of age. Explanatory variables considered for primary objectives include cord blood cytokines, maternal cytokines and pollutants.

Cord blood cytokines consisted of il4phacord, il5phacord, il10phacord, il12phacored and INFgphacord while maternal cytokines comprised of il4phamaternal, il5phamaternal, il10phamaternal, il12phamaternal and INFgphamaternal. Among the pollutants were Lead in maternal blood serum (Pbblood), Cadmium in maternal blood serum (Cdblood), Lead in placenta (Pbplacenta), Cadmium in placenta (Cdplacenta), sum of chlorinated benzenes in maternal blood serum *(MBS-sum1)*, sum of Polychlorinated biphenyls (PCBs) in maternal blood serum *(MBS-sum2)*, sum of Organochlorine insecticides in maternal blood serum*(MBS-sum3)*,sum of chlorinated benzenes in placenta*(PLAT-sum1)* , sum of Polychlorinated biphenyls (PCBs) in placenta*(PLAT-sum2)* , sum of Organochlorine insecticides in placenta *(PLAT-sum3)*, sum of chlorinated benzenes in breast milk *(BM-sum1)*, sum of Polychlorinated biphenyls (PCBs) in breast milk*(BM-sum2)* , and sum of Organochlorine insecticides in breast milk *(BM-sum3)*.

Different statistical methodologies were used to analyze the data inorder to draw conclusions based on the objectives of the study. Techniques used to analyze the data included multiple regression analysis, multiple logistic regression analysis, multivariate logistic regression analysis and Reciever Operating Characteristic (ROC) curves. Results obtained using these analytical techniques are discussed in the subsequent sections.

Associations between cord blood cytokines and allergic outcomes

A model cumulating allergies disclosed that cytokine il4phacord increased the probability of having atleast one of the four allergies and also predicted the probability of having atleast one of the four allergies in child.

It was observed from Multivariate models that elevated levels of il4phacord, il5phacord and il10phacord increased the probability of manifesting all four allergies. This is expected since allergic subjects are more likely to have elevated levels of the Th2 cytokines il4, il5 and il10.This result is consistent with findings from (Duramand *et al*., (2006)) who found out that allergy

outcomes, Asthma in particular, in children at 24 months of age were associated with elevated levels of Th2 cytokines (il4, il5 and il10). Cytokines il4phacord, il5phacord and il10phacord predicted Atopic eczema but they did not predict Otherdermatitis, specific IgE for inhalant allergies (PhadCHinhalant) and specific IgE for food allergies (fx5CHfood).

Associations between maternal cytokines and allergic outcomes
Using a model cumulating allergies, children in Mol had higher chances of having atleast one allergy than Stara Lubovna. The variable region predicted the probability of having atleast one allergy in child.

Results from multivariate models revealed that, Slovakia had lower chances of having allergies than Belgium. This result is in line with exploratory data analysis where children in Belgium were found to have the highest prevalence of Atopic eczema. In addition, Belgium had the highest number of allergic and atopic mothers. Increased levels of INFgmaternal cytokines increased chances of all allergies. INFgmaternal cytokines and country predicted Atopic eczema and food allergies but they did not predict Otherdermatitis and inhalant allergies.

Associations between pollutants and allergic outcomes
A model cumulating allergies demonstrated that, pollutants BM-sum3 and Pbplacenta increased the probability of manifesting atleast one allergy while increased levels of PLAT-sum3 and Pbblood reduced the probability of manifesting atleast one allergy in child. Pollutants BM-sum3, Pbplacenta, PLAT-sum3 and Pbblood predicted the probability of manifesting atleast one allergy in child.

Results from multivariate models showed that, increased levels of the sum of organochlorine insecticides in breast milk (BM-sum3), Cadmium in placenta (Cdplacenta), sum of Organochlorine insecticides in maternal blood (MBS-sum3) increased the probability of developing all four allergies. These findings are expected and tally with findings of Daniel *et al*., (2001) who found out that exposure to Polychlorinated biphenyls and Organochlorines suppress immune responses which favour allergic outcomes. Higher levels of Lead in maternal blood serum (Pbblood), sum of Chlorinated benzenes in maternal blood serum (MBS-sum1), sum of PCBs in maternal blood serum (MBS-sum2), sum of PCBs in placenta (PLAT-sum2) and sum of PCBs in breast milk (BM-sum2) reduce chances of all four allergies in child at 18 months. Atopic eczema was predicted by these pollutants whereas Otherdermatitis, food allergies and inhalant allergies were not predicted.

Models fitted on complete case data sets are valid only when data are missing completely at random (MCAR). Models fitted on imputed data sets are valid if data are missing at random (MAR). Precisely multiple imputation is an appealing alternative.

7 Conclusion and Recommendation

Conclusion

Neonatal (cord blood) cytokines, especially il4phacord, il5phacord and il10phacord and, maternal cytokines, INFgmaternal above others were found to be early predictive biomarkers of allergic outcomes in child at 18 months.

Allergic outcomes in child at 18months are affected sum of organochlorine insecticides in breast milk (BM-sum3), Cadmium in placenta (Cdplacenta), sum of Organochlorine insecticides in maternal blood (MBS-sum3),Lead in maternal blood serum (Pbblood), sum of Chlorinated benzenes in maternal blood serum (MBS-sum1), sum of PCBs in maternal blood serum (MBS-sum2), sum of PCBs in placenta (PLAT-sum2) and sum of PCBs in breast milk (BM-sum2).

Slovakia had lower chances of having allergies than Belgium and Stara Lubovna had lower chances of allergy than Mol. Also, the variable region predicted the probability of having allergy in child at 18 moths of age.

Recommendation

Only multiple regression analysis was done for the secondary objective of the study due to lack of enough time. Since many measurements were taken from the same individuals, we recommend that multivariate analysis should be done for the secondary objective inorder to capture the associations among responses.

Data about hospitals needs to be provided in future since hospitals in different environmental exposures are likely to impact on the participants differently.

Only mothers and babies participated in our study. However, there is evidence from various studies that allergy can result from genetic background of an individual (Von., (2004)). Therefore, participation of fathers in such studies needs be considered.

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9 Appendix

Appendix A: Data and Variable description

Appendix A1: Biomarkers of exposure

Table A1.1: List of metals and organic compounds

Biomarker	Variable name in data set		
Metals	**Maternal blood**	**Placenta**	**Breast milk**
Pb	Pbblood	Pbplacenta	
Cd	Cdblood	Cdplacenta	
Organic compounds			
1. Chlorinated benzenes			
1,3 DiClB	1,3 DiClBblood	1,3 DiClBplacenta	1,3 DiClBbreast
1,4 DiClB	1,4 DiClBblood	1,4 DiClBplacenta	1,4 DiClBbreast
1,2 DiClB	1,2 DiClBblood	1,2 DiClBplacenta	1,2 DiClBbreast
1,3,5 TrClB	1,3,5 TrClBblood	1,3,5 TrClBplacenta	1,3,5 TrClBbreast
1,2,4 TrClB	1,2,4 TrClBblood	1,2,4 TrClBplacenta	1,2,4 TrClBbreast
1,2,3 TrClB	1,2,3 TrClBblood	1,2,3 TrClBplacenta	1,2,3 TrClBbreast
TeClB	TeClBblood	TeClBplacenta	TeClBbreast
1.2.3.4 TeClB	1.2.3.4 TeClBblood	1.2.3.4 TeClBplacenta	1.2.3.4TeClBbreast
PeClB	PeClBblood	PeClBplacenta	PeClBbreast
HCB	HCBblood	HCBplacenta	HCBbreast
2. Organochlorine insecticides			
alfa HCH	alfa HCHblood	alfa HCHplacenta	alfa HCHbreast
beta HCH	beta HCHblood	beta HCHplacenta	beta HCHbreast
Lindan	Lindanblood	Lindanplacenta	Lindanbreast
delta HCH	delta HCHblood	delta HCHplacenta	delta HCHbreast
DDE	DDEblood	DDEplacenta	DDEbreast
DDT	DDTblood	DDTplacenta	DDTbreast
3. **(PCBs)**			
PCB 28	PCB 28blood	PCB 28placenta	PCB 28placenta
PCB 52	PCB 52blood	PCB 52placenta	PCB 52placenta
PCB 101	PCB 101blood	PCB 101placenta	PCB 101placenta
PCB 118	PCB 118blood	PCB 118placenta	PCB 118placenta
PCB 153	PCB 153blood	PCB 153placenta	PCB 153placenta
PCB 138	PCB 138blood	PCB 138placenta	PCB 138placenta
PCB 180	PCB 180blood	PCB 180placenta	PCB 180placenta

Table A1.2: Description of metals and organic compounds

Metals

Pb – Lead
Cd – Cadmium
Pbblood - lead in maternal blood
Cdblood - cadmium in maternal blood
Pbplacenta - lead in placenta
Cdplacenta - cadmium in placenta

Organic compounds

1. Chlorinated benzenes
1,3 DiClB - 1,3-dichlorobenzene in maternal blood serum
1,4 DiClB -1,4-dichlorobenzene in maternal blood serum
1,2 DiClB -1,2-dichlorobenzene in maternal blood serum
1,3,5 TrClB -1,3,5-trichlorobenzene in maternal blood serum
1,2,4 TrClB -1,2,4-trichlorobenzene in maternal blood serum
1,2,3 TrClB -1,2,3-trichlorobenzene in maternal blood serum
TeClB – sum(1,2,3,5+1,2,4,5)tetrachlorobenzene in maternal blood serum
1,2,3,4 TeClB - 1,2,3,4-tetrachlorobenzene in maternal blood serum
PeClB – pentachlorobenzene in maternal blood serum
HCB – hexachlorobenzene in maternal blood serum

2. Organochlorine insecticides
alfa HCH – alpha-hexachlorocyclohexane in maternal blood serum
beta HCH – beta-hexachlorocyclohexane in maternal blood serum
Lindan – gamma-hexachlorocyclohexane in maternal blood serum
delta HCH – delta-hexachlorocyclohexane in maternal blood serum
DDE-1′,1 – dichloro-2,2-bis(p-chlorophenyl)ethylene in maternal blood serum
DDT-1,1,1 - trichloro-2,2-bis(p-chlorophenyl)ethane in maternal blood serum

3. Polychlorinated biphenyls (PCBs)
PCB 28 – (2,4,4′- trichlorobiphenyl) in maternal blood serum
PCB 52 – (2,2′,5,5′- tetrachlorobiphenyl) in maternal blood serum
PCB 101 – (2,2′,4,5,5′- pentachlorobiphenyl)in maternal blood serum
PCB 118 – (2,3′,4,4′,5 – pentachlorobiphenyl) in maternal blood serum
PCB 153 – (2,2′,3,4,4′,5′- hexachlorobiphenyl) in maternal blood serum
PCB 138 – (2,2′,4,4′,5,5′- hexachlorobiphenyl) in maternal blood serum
PCB 180 – (2,2′,3,4,4′,5,5′- heptachlorobiphenyl) in maternal blood serum

Appendix A2: Biomarkers of effect

Table A2.1: List of biomarkers of effect

Biomarker	Variable name in data set
Anti-oxidants	
1. Maternal blood	
Catalase	CATblood
Superoxide dismutase (SOD)	SODblood
Glutathione peroxidase (GPx)	GPXblood
Glutathione (GST)	GSTblood
nonproteic SH-groups (SHnp)	SHnpblood
proteic SH-groups (SH total)	SHtotalblood
2. Placenta	
Catalase	CATplacenta
Superoxide dismutase (SOD)	SODplacenta
Glutathione peroxidase (GPx)	GPXplacenta
Glutathione (GST)	GSTplacenta
Glutathione reductase (GOR)	GORplacenta
nonproteic SH-groups (SHnp)	SHnpplacenta
total proteic SH-groups (SH total)	SHtotalplacenta
Maternal peripheral blood cytokines	
Interferon-g	INF-gphamaternal
interleukin-4	IL-4phamaternal
interleukin-5	IL-5phamaternal
interleukin-10	IL-10phamaternal
interleukin-12	IL-12phamaternal
Cord blood cytokines	
1. After PHA stimulation	
Interferon-g	INF-gphacord
interleukin-4	il-4phacord
interleukin-5	il-5phacord
interleukin-10	il-10phacord
interleukin-12	il-12phacord
2. After lactalbumin stimulation	
Interferon-g	IFN-gLAcord
interleukin-4	il-4LAcord
interleukin-5	il-5LAcord
interleukin-10	il-10LAcord
interleukin-12	il-12LAcord
3. After ovalbumin stimulation	
Interferon-g	IFN-gOAcord
interleukin-4	il-4OAcord
interleukin-5	il-5OAcord
interleukin-10	il-10OAcord
interleukin-12	il-12OAcord
4. After house dust mite stimulation	
Interferon-g	IFN-gHDMcord
interleukin-4	il-4HDMcord
interleukin-5	il-5HDMcord

interleukin-10	il-10HDMcord
interleukin-12	il-12HDMcord
Placenta cytokines	
interleukin-4	il4placenta
interleukin-5	il5placenta
interleukin-10	il10placenta
Interferon-g	INF-gplacenta
tnfplacenta	tnfplacenta
Child peripheral blood cytokines at 18 months	
Interferon-g	INF-gchild
interleukin-2	il-2child
interleukin-4	il-4child
interleukin-5	il-5child
interleukin-10	il-10child
Clinical exam of child at 18 months	
Weight [Kg]	Weight [Kg]
Length [cm]	Length [cm]
atopic eczema (0=no; 1=yes)	Atopiceczema
other dermatitis (0=no; 1= yes)	Otherdet
Specific IgE of child at 18 months	
specific IgE for inhalent allergies in child blood	PhadCH inhalant
specific IgE for food allergies in child blood	fx5CH food
Immune status of mother	
Allergic history of mother	Allergicmother
total IgE in maternal and cord blood	Total IgE
Specific IgE (Phadiatop) in maternal blood	Specific IgE
atopic/nonatopic status of mother (Y=yes, N=no, NA=doubtful)	Atopic/nonAtopic
Low level cord blood IgE	Lowcordblood IgE

Appendix B: Exploratory data analysis

Table B1: Proportions of Missing observations in biomarkers of exposure

Variables	No. of missing observations	No. of observations with all information	Proportion of missing observations
Maternal blood			
Metals			
Pbblood	15	485	0.03
Cdblood	15	485	0.03
Organic compounds			
1.Chlorinated benzenes			
1,3 DiClBblood	30	470	0.06
1,4 DiClBblood	30	470	0.06
1,2 DiClBblood	30	470	0.06
1,3,5 TrClBblood	30	470	0.06
1,2,4 TrClBblood	30	470	0.06
1,2,3 TrClBblood	30	470	0.06
TeClBblood	30	470	0.06
1.2.3.4 TeClBblood	30	470	0.06
PeClBblood	30	470	0.06
HCBblood	30	470	0.06
2.Polychlorinated biphenyls (PCBs)			
PCB 28blood	30	470	0.06
PCB 52blood	30	470	0.06
PCB 101blood	30	470	0.06
PCB 118blood	30	470	0.06
PCB 153blood	30	470	0.06
PCB 138blood	30	470	0.06
PCB 180blood	30	470	0.06
3.Organochlorine insecticides			
alfa HCHblood	30	470	0.06
beta HCHblood	30	470	0.06
Lindanblood	30	470	0.06
delta HCHblood	30	470	0.06
DDEblood	30	470	0.06

DDTblood	30	470	0.06
Placenta			
1.Metals			
Pbplacenta	20	480	0.04
Cdplacenta	20	480	0.04
Organic compounds			
1.Chlorinated benzenes			
1,3 DiClBplacenta	1	499	0.002
1,4 DiClBplacenta	1	499	0.002
1,2 DiClBplacenta	1	499	0.002
1,3,5 TrClBplacenta	1	499	0.002
1,2,4 TrClBplacenta	1	499	0.002
1,2,3 TrClBplacenta	1	499	0.002
TeClBplacenta	1	499	0.002
1.2.3.4 TeClBplacenta	1	499	0.002
PeClBplacenta	1	499	0.002
HCBplacenta	1	499	0.002
2.Polychlorinated biphenyls (PCBs)			
PCB 28placenta	1	499	0.002
PCB 52placenta	1	499	0.002
PCB 101placenta	1	499	0.002
PCB 118placenta	1	499	0.002
PCB 153placenta	1	499	0.002
PCB 138placenta	1	499	0.002
PCB 180placenta	1	499	0.002
3.Organochlorine insecticides			
alfa HCHplacenta	1	499	0.002
beta HCHplacenta	1	499	0.002
Lindanplacenta	1	499	0.002
delta HCHplacenta	1	499	0.002
DDEplacenta	1	499	0.002
DDTplacenta	1	499	0.002
Breast milk			

1.Chlorinated benzenes			
1,3 DiClBbreast	216	284	0.432
1,4 DiClBbreast	216	284	0.432
1,2 DiClBbreast	216	284	0.432
1,3,5 TrClBbreast	216	284	0.432
1,2,4 TrClBbreast	216	284	0.432
1,2,3 TrClBbreast	216	284	0.432
TeClBbreast	216	284	0.432
1.2.3.4 TeClBbreast	216	284	0.432
PeClBbreast	216	284	0.432
HCBbreast	216	284	0.432
2.Polychlorinated biphenyls (PCBs)			
PCB 28breast	216	284	0.432
PCB 52breast	216	284	0.432
PCB 101breast	216	284	0.432
PCB 118breast	216	284	0.432
PCB 153breast	216	284	0.432
PCB 138breast	216	284	0.432
PCB 180breast	216	284	0.432
3.Organochlorine insecticides			
alfa HCHbreast	216	284	0.432
beta HCHbreast	216	284	0.432
Lindanbreast	216	284	0.432
delta HCHbreast	216	284	0.432
DDEbreast	216	284	0.432
DDTbreast	216	284	0.432

Table B2: Proportions of missing observations in biomarkers of effect

Variables	No. of missing observations	No. of observations with all information	Proportions of missing observations
Maternal blood cytokines			
INFgphamaternal	225	275	0.45
Il4phamaternal	213	287	0.426
Il5phamaternal	206	294	0.412
Il10phamaternal	306	194	0.612
Il12phamaternal	317	183	0.634
Cord blood cytokines			
il4phacord	267	233	0.534
il5phacord	228	272	0.456
il10phacord	280	220	0.56
il12phacord	316	184	0.632
IFNgphacord	268	232	0.536
Child cytokines			
INFgchild	422	78	0.844
il2child	422	78	0.844
il4child	422	78	0.844
il5child	422	78	0.844
il10child	422	78	0.844
Placenta cytokines			
INFgplacenta	326	174	0.652
il5placenta	326	174	0.652
il4placenta	326	174	0.652
il10placenta	326	174	0.652
tnfplacenta	327	173	0.654
Child 18months			
Atopiceczema	154	346	0.308
Otherdet	154	346	0.308
PhadCHinhalant	169	331	0.338
fx5CHfood	168	332	0.336
Immunity mother			
Allergicmother	1	499	0.002
Atopic/nonAtopic	10	490	0.02
SpecificIgE	7	493	0.014
TotalIgE	7	493	0.014

Table B3: Overall Concentration of toxic metals (µg/l)

Variable	Mean	Std Dev	Min	Max
Pbblood	33.303	22.728	9.990	198.790
Cdblood	0.491	1.075	0.050	18.594
Pbplacenta	0.030	0.070	0.006	0.829
Cdplacenta	0.008	0.007	0.001	0.087

Table B4: Correlations *(P-values)* between toxic metals

Variable	Pbblood	Cdblood	Pbplacenta	Cdplacenta
Pbblood	1			
Cdblood	0.418 *(<.0001)*	1		
Pbplacenta	0.641 *(<.0001)*	0.421 *(<.0001)*	1	
Cdplacenta	0.263 *(<.0001)*	0.547 *(<.0001)*	0.306 *(<.0001)*	1

Table B5: Concentration of metals by country (µg/l)

Country	Maternal blood				Placenta			
	Pbblood		Cdblood		Pbplacenta		Cdplacenta	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
Slovakia	24.543	9.826	0.266	0.223	0.016	0.012	0.008	0.007
Romania	49.950	27.411	0.799	1.671	0.055	0.109	0.009	0.005
Belgium	19.774	8.269	0.367	0.182	0.014	0.011	0.009	0.010

Table B6: Ranges of concentration of toxic metals by country (µg/l)

Variable	Country					
	Slovakia		Romania		Belgium	
	Min	Maxi	Min	Maxi	Min	Max
Pbblood	11.710	94.860	12.990	94.860	9.990	66.440
Cdblood	0.05 0	1.892	0.169	18.594	0.137	0.911
Pbplacenta	0.006	0.131	0.010	0.829	0.006	0.109
Cdplacenta	0.001	0.087	0.002	0.028	0.002	0.075

Table B7: Ranges of Concentrations of metals by region (µg/l)

Variable	Slovakia				Romania				Belgium	
	Brastislava		Stara Lubovna		Bucharest		Giurgiu		Mol	
	Max	Min	Min	Max	Min	Max	Min	Max	Min	Max
Pbblood	11.710	52.470	13.430	94.860	18.320	126.100	12.990	198.790	9.990	66.440
Cdblood	0.050	1.892	0.058	1.366	0.169	18.594	0.196	10.865	0.137	0.911
Pbplacenta	0.006	0.045	0.008	0.131	0.010	0.104	0.010	0.829	0.006	0.109
Cdplacenta	0.001	0.087	0.002	0.023	0.002	0.019	0.003	0.028	0.002	0.075

Table B8: Concentration of organic compounds over all countries (ng/g fat)

Variable	Mean	Std Dev	Min	Max
MBS-sum1	309.516	1006	16.835	15304
MBS-sum2	195.689	199.586	10.457	1576
MBS-sum3	824.519	1160	8.6280	14003
PLAT-sum1	231.404	1023	18.071	21257
PLAT-sum2	239.336	2220	4.9295	49485
PLAT-sum3	824.092	2059	19.383	40416
BM-sum1	183.006	192.280	18.024	1289
BM-sum2	277.663	235.088	12.221	2912
BM-sum3	2311	2806	99.344	20030

Table B9: correlations *(P-values)* among organic compounds (Overall)

Variable	MBS-sum1	MBS-sum2	MBS-sum3	PLAT-sum1	PLAT-sum2	PLAT-sum3	BM-sum1	BM-sum2	BM-sum3
MBS-sum1	1								
	0.408								
MBS-sum2	*(<.001)*	1							
	0.177	-0.056							
MBS-sum3	*(<.001)*	*(0.226)*	1						
	0.052	-0.177	0.236						
PLAT-sum1	*(0.264)*	*(0.001)*	*(<.001)*	1					
	0.037	0.375	-0.091	0.275					
PLAT-sum2	*(0.424)*	*(<.001)*	*(0.048)*	*(<.001)*	1				
	-0.136	-0.405	**0.694**	0.491	0.113				
PLAT-sum3	*(0.003)*	*(<.001)*	***(<.001)***	*(<.001)*	*(0.011)*	1			
	0.089	-0.083	0.296	**0.518**	0.191	0.421			
BM-sum1	*(0.145)*	*(0.175)*	*(<.001)*	***(<.001)***	*(0.001)*	*(<.001)*	1		
	-0.013	0.124	0.274	0. 365	**0.517**	0.406	0.454		
BM-sum2	*(0.830)*	*(0.043)*	*(<.001)*	*(<.001)*	***(<.001)***	*(<.001)*	*(<.001)*	1	
	-0.072	-0.362	**0.753**	0.403	0.012	**0.906**	0.488	**0.511**	
BM-sum3	*(0.238)*	*(<.001)*	***(<.001)***	*(<.001)*	*(0.84)*	***(<.001)***	*(<.001)*	***(<.001)***	1

Table B10: Concentration of organic compounds by country (ng/g fat)

Variable	Country					
	Slovakia		Romania		Belgium	
	Mean	Std Dev	Mean	Std Dev	Mean	Std Dev
MBS-sum1	252.343	843.218	315.805	780.852	401.112	1526
MBS-sum2	224.188	196.342	114.737	161.435	297.108	211.529
MBS-sum3	299.871	233.077	1526	1551	447.501	350.923
PLAT-sum1	301.322	1522	245.276	527.162	63.962	108.739
PLAT-sum2	301.322	1522	118.512	127.453	104.742	87.311
PLAT-sum3	483.968	2923	1511	1110	137.432	123.327
BM-sum1	197.341	191.328	212.533	184.401	98.090	188.444
BM-sum2	297.584	189.744	316.706	293.761	163.770	87.799
BM-sum3	708.730	452.550	4504	3036	448.754	449.027

Table B11: Ranges of concentration of organic compounds by country (ng/g fat)

Variable	Country					
	Slovakia		Romania		Belgium	
	Min	Maxi	Min	Maxi	Min	Max
MBS-sum1	25.293	11302	20.538	6494	16.835	15304
MBS-sum2	10.457	1448	11.343	1576	10.638	1190
MBS-sum3	22.009	1666	226.764	14003	8.628	1634
PLAT-sum1	18.908	21257	30.846	6890	18.071	1016
PLAT-sum2	10.517	49485	12.446	1021	4.930	530.530
PLAT-sum3	46.441	40416	143.737	8931	19.383	714.459
BM-sum1	26.205	1289	18.393	1173	18.024	1283
BM-sum2	12.221	1300	19.964	2912	49.636	528.426
BM-sum3	122.791	2339	472.324	20030	99.345	2738

Table B12: Correlations *(P-values)* between pairs of organic compounds in Slovakia

Variable	MBS-sum1	MBS-sum2	MBS-sum3	PLAT-sum1	PLAT-sum2	PLAT-sum3	BM-sum1	BM-sum2	BM-sum3
MBS-sum1	1								
MBS-sum2	0.443 *(<.001)*	1							
MBS-sum3	0.473 *(<.001)*	**0.516** ***(<.001)***	1						
PLAT-sum1	0.362 *(<.001)*	0.087 *(0.245)*	0.155 *(0.038)*	1					
PLAT-sum2	0.216 *(0.003)*	0.453 *(<.001)*	0.282 *(0.001)*	0.341 *(<.001)*	1				
PLAT-sum3	0.069 *(0.354)*	0.122 *(0.102)*	0.522 *(<.001)*	0.476 *(<.001)*	**0.502** ***(<.001)***	1			
BM-sum1	**0.525** ***(<.001)***	0.283 *(0.008)*	0.275 *(0.009)*	**0.629** ***(<.001)***	0.321 *(0.001)*	0.273 *(0.006)*	1		
BM-sum2	0.292 *(0.006)*	**0.678** ***(<.001)***	0.255 *(0.016)*	0.239 *(0.016)*	**0.622** ***(<.001)***	0.227 *(0.023)*	0.492 *(<.001)*	1	
BM-sum3	0.268 *(0.012)*	0.455 *(<.001)*	**0.587** ***(<.001)***	0.292 *(0.003)*	0.327 *(<.001)*	**0.652** ***(<.001)***	**0.567** ***(<.001)***	**0.576** ***(<.001)***	1

Table B13: Correlations *(P-values)* between pairs of organic compounds in Romania

Variable	MBS-sum1	MBS-sum2	MBS-sum3	PLAT-sum1	PLAT-sum2	PLAT-sum3	BM-sum1	BM-sum2	BM-sum3
MBS-sum1	1								
MBS-sum2	0.295 *(<.001)*	1							
MBS-sum3	0.305 *(<.001)*	**0.560** ***(<.001)***	1						
PLAT-sum1	0.119 *(0.101)*	0.049 *(0.502)*	0.037 *(0.615)*	1					
PLAT-sum2	-0.015 *(0.841)*	0.462 *(<.001)*	0.254 *(<.001)*	0.229 *(0.001)*	1				
PLAT-sum3	0.141 *(0.053)*	0.229 *(0.002)*	**0.654** ***(<.001)***	0.128 *(0.071)*	0.427 *(<.001)*	1			
BM-sum1	0.058 *(0.524)*	0.069 *(0.453)*	0.231 *(0.011)*	0.184 *(0.041)*	0.038 *(0.674)*	0.183 *(0.042)*	1		
BM-sum2	-0.077 *(0.403)*	0.261 *(0.004)*	0.258 *(0.004)*	0.124 *(0.170)*	0.456 *(<.001)*	0.320 *(<.001)*	0.203 *(0.023)*	1	
BM-sum3	0.067 *(0.462)*	0.250 *(0.006)*	0.543 *(<.001)*	0.068 *(0.451)*	0.344 *(<.001)*	**0.548** ***(<.001)***	0.279 *(0.002)*	**0.596** ***(<.001)***	1

Table B14: Correlations *(P-values)* between pairs of organic compounds in Belgium

Variable	MBS-sum1	MBS-sum2	MBS-sum3	PLAT-sum1	PLAT-sum2	PLAT-sum3	BM-sum1	BM-sum2	BM-sum3
MBS-sum1	1								
MBS-sum2	0.480 *(<.001)*	1							
MBS-sum3	**0.522** ***(<.001)***	**0.644** ***(<.001)***	1						
PLAT-sum1	-0.052 *(0.607)*	0.259 *(0.009)*	0.022 *(0.827)*	1					
PLAT-sum2	-0.088 *(0.383)*	0.220 *(0.028)*	-0.102 *(0.310)*	0.404 *(<.001)*	1				
PLAT-sum3	-0.090 *(0.372)*	0.030 *(0.767)*	-0.084 *(0.408)*	0.393 *(<.001)*	0.522 *(<.001)*	1			
BM-sum1	0.115 *(0.381)*	0.070 *(0.598)*	-0.009 *(0.947)*	0.214 *(0.101)*	0.267 *(0.039)*	0.161 *(0.219)*	1		
BM-sum2	0.128 *(0.331)*	0.196 *(0.134)*	0.117 *(0.372)*	0.351 *(0.006)*	**0.584** ***(<.001)***	**0.500** ***(<.001)***	0.269 *(0.038)*	1	
BM-sum3	0.026 *(0.846)*	0.054 *(0.681)*	0.088 *(0.505)*	0.207 *(0.112)*	0.249 *(0.055)*	**0.821** ***(<.001)***	0.194 *(0.138)*	0.476 *(<.001)*	1

Table B14a: Ranges of Concentrations of organic compounds by region (ng/g fat)

Variable	Romania				Belgium	
	Bucharest		Giurgiu		Mol	
	Min	Max	Min	Max	Min	Max
MBS-sum1	24.759	1727	20.538	6494	16.834	15304
MBS-sum2	15.905	1576	11.343	1018	10.638	1190
MBS-sum3	243.410	14003	226.7639	9272	8.628	1634
PLAT-sum1	30.845	6890	35.363	1225	18.071	1016
PLAT-sum2	12.446	975.762	15.206	1021	4.929	530.53
PLAT-sum3	143.737	7480	456.213	8931	19.383	714.459
BM-sum1	48.692	1173	18.393	1075	18.024	1283
BM-sum2	94.293	1042	19.964	2912	49.636	528.426
BM-sum3	907.408	20030	472.324	19339	99.345	2738

Table B14b: Ranges of Concentrations of organic compounds by region (ng/g fat)

Variable	Slovakia			
	Brastislava		Stara Lubovna	
	Max	Min	Min	Max
MBS-sum1	25.293	11302	27.072	981.925
MBS-sum2	10.457	1276	19.893	1448
MBS-sum3	22.009	1666	32.933	1163
PLAT-sum1	24.176	21257	18.908	910.076
PLAT-sum2	50.373	49485	10.517	742.097
PLAT-sum3	46.441	40416	60.591	1012
BM-sum1	26.205	1289	53.089	942.301
BM-sum2	69.897	1300	12.221	1003
BM-sum3	122.791	2339	130.228	1339

Table B15: Correlations among maternal blood cytokines

Variables	INFgpha	Il4pha	Il5pha	Il10pha	Il12pha
INFgpha	1				
Il4pha	0.768 *(<.001)*	1			
Il5pha	0.805 *(<.001)*	0.800 *(<.001)*	1		
Il10pha	0.809*(<.001)*	0.685 *(<.001)*	0.808 *(<.001)*	1	
Il12pha	0.869 *(<.001)*	0.654 *(<.001)*	0.696 *(<.001)*	0.722 *(<.001)*	1

Table B16: Correlations among cord blood cytokines

Variables	il4pha	il5pha	il10pha	il12pha	IFNgpha
il4pha	1				
il5pha	0.609 *(<.001)*	1			
il10pha	0.815 *(<.001)*	0.688 *(<.001)*	1		
il12pha	0.597 *(<.001)*	0.632 *(<.001)*	0.691 *(<.001)*	1	
IFNgpha	0.734 *(<.001)*	0.705 *(<.001)*	0.833 *(<.001)*	0.734 *(<.001)*	1

Table B17: Correlations among child blood cytokines

Variables	INFg	il2	il4	il5	il10
INFg	1				
il2	0.653 *(<.001)*	1			
il4	0.378 *(0.006)*	0.599 *(<.001)*	1		
il5	0.512 *(<.001)*	0.558 *(<.001)*	0.744 *(<.001)*	1	
il10	0.658 *(<.001)*	0.579 *(<.001)*	0.454 *(<.001)*	0.574 *(<.001)*	1

Table B18: Correlations *(P-values)* among placenta blood cytokines

Variable	INFg	il5	il4p	il10	tnf
INFg	1				
il4	0.396 *(<.001)*	1			
il5	0.418 *(<.001)*	0.336 *(<.001)*	1		
il10	0.299 *(<.001)*	0.011 *(0.889)*	-0.003 *(0.973)*	1	
tnf	0.196 *(0.001)*	-0.178 *(0.019)*	-0.041 *(0.597)*	0.641*(<.001)*	1

Table B19: Frequencies within allergy measures over all countries

Measure of allergy	No. of children (%)
Atopiceczema	
No	292 (84.4)
Yes	54 (15.6)
Otherdet	
No	329 (95.1)
Yes	17 (4.9)
PhadCHinhalant	
No	328 (99.1)
Yes	3 (0.9)
fx5Chfood	
No	315 (94.9)
Yes	17 (5.1)

Table B20: Frequencies within allergy measures by country

	Country		
	Slovakia	Romania	Belgium
Measure of allergy	No. of children (%)		
Atopiceczema			
No	150 (86.2)	105 (89.7)	37 (67.3)
Yes	24 (13.8)	12 (10.3)	18 (32.7)
Otherdet			
No	166 (95.4)	110 (94.0)	53 (96.4)
Yes	8 (4.6)	7 (6.0)	2 (3.6)
PhadCHinhalant			
No	171 (100)	86 (97.7)	71 (98.6)
Yes	0	2 (2.3)	1 (1.4)
fx5Chfood			
No	160 (93.6)	87 (97.6)	68 (94.4)
Yes	11 (6.4)	2 (2.4)	4 (5.6)

Table B21: Number of mothers (%) with allergy over all countries

Measure of immunity	Levels	No. of mothers (%)
Allergicmother	No	246 (49.3)
	Yes	253 (50.7)
Atopic_nonAtopic	No	147 (30.0)
	Yes	98 (20.0)
	Doubtful	245 (50.0)

Table B22: Number of mothers (%) with allergy by country

Measure of immunity	Levels	Country		
		Slovakia	Romania	Belgium
Allergicmother	No	94 (47.2)	123 (61.5)	29 (29.0)
	Yes	105 (52.8)	77 (38.5)	71 (71.0)
Atopic_nonAtopic	No	59(29.8)	69 (35.8)	19 (19.2)
	Yes	40 (20.2)	22 (11.4)	36 (36.4)
	Doubtful	99 (50.0)	102 (52.9)	44 (44.4)

Table B23: TotalIgE over all countries, by country & by region (kU/l)

	Mean	Std Dev	Min	Max
Over all	422.921	793.536	0.360	5140
By Country				
Slovakia	359.354	761.314	1.110	5140
Romania	503.308	883.790	0.360	5023
Belgium	394.907	654.199	2.000	3147
By region				
Slovakia				
Brastislava	281.978	611.777	2.000	4986
Stara Lubovna	436.730	882.432	1.110	5140
Romania				
Bucharest	276.868	589.054	76.355	3275
Giurgiu	727.414	1057	2.000	5023
Belgium				
Mol	394.907	654.199	2.000	3147

Appendix C: Tables from statistical models

Appendix C1: Areas under curves from multiple logistic models

Table C1.1: Area under curve (AUC), STD and 95% (C.I.)

Allergic outcome	AUC	STD	95% (C.I.)
Atopic eczema	0.654	0.035	(0.584, 0.724)
Otherdermatitis	0.549	0.067	(0.414, 0.684)
PhadCHinhalant	0.987	0.031	(0.925, 1.000)
fx5CHfood	0.138	0.030	(0.078, 0.199)

Table C1.2: Area under curve (AUC), STD and 95% C.I.

Allergic outcome	AUC	STD	95% (C.I.)
Atopic eczema	0.656	0.035	(0.586, 0.726)
Otherdermatitis	0.801	0.060	(0.681, 0.921)
PhadCHinhalant	0.979	0.038	(0.902, 1.000)
fx5CHfood	0.584	0.072	(0.440, 0.727)

Table C1.3: Area under curve (AUC), STD and 95% (C.I.)

Allergic outcome	AUC	STD	95% (C.I.)
Atopic eczema	0.697	0.034	(0.628, 0.766)
Otherdermatitis	0.580	0.068	(0.444, 0.716)
PhadCHinhalant	0.972	0.044	(0.885, 1.000)
fx5CHfood	0.756	0.067	(0.622, 0.890)

Appendix C2: Multiple regression models of the secondary objective

Table C2.1: Relationship between pollutants and, Allergic history, Atopic status & TotalIgE of the mother

Response	Parameter	Estimate (s.e)	P-value	OR (95% C.I.)
Allergic history	Intercept	0.497 (0.175)	0.005	
	Pbblood	-0.014 (0.005)	0.002	0.986 (0.977,0.995)
	BM-sum3	-0.071 (0.027)	0.009	0.931 (0.883,0.982)
Atopic status	Intercept	-1.458 (0.129)	<.001	
	MBS-sum2	-0.100 (0.036)	0.005	0.904 (0.843,0.970)
	BM-sum2	0.058 (0.024)	0.015	1.060 (1.012,1.111)
	BM-sum3	-0.119 (0.042)	0.005	0.888 (90.817,0.964)
TotalIgE	Intercept	407.023 (35.135)	<.001	
	PLAT-sum1	11.736 (6.433)	0.069	-
	PLAT-sum2	-15.507 (8.193)	0.059	-
	BM-sum2	21.544 (7.367)	0.004	-
	BM-sum3	-39.054 (11.062)	0.001	-

Table C2.2: Effect of pollutants on INFgplacenta

Parameter	Estimate (s.e)	P-value
Intercept	2.742 (0.146)	<.001
Pbblood	-0.015 (0.004)	<.001
Pbplacenta	34.231 (1.218)	<.001
MBS-sum2	0.046 (0.021)	0.028
MBS-sum3	-0.079 (0.029)	0.007
PLAT-sum1	0.057 (0.017)	<.001
PLAT-sum2	-0.201 (0.021)	<.001
PLAT-sum3	0.249 (0.031)	<.001
BM-sum3	-0.097 (0.025)	0.001

Table C2.3: Effect of pollutants on il4placenta

Parameter	Estimate (s.e)	P-value
Intercept	0.769 (0.119)	<.001
Pbblood	-0.012 (0.003)	<.001
Pbplacenta	25.188 (0.727)	<.001
PLAT-sum1	0.064 (0.010)	<.001
PLAT-sum2	-0.092 (0.013)	<.001
PLAT-sum3	0.033 (0.018)	0.059
BM-sum1	-0.031(0.009)	<.001
BM-sum2	0.023 (0.010)	0.022
BM-sum3	-0.041(0.017)	0.015

Table C2.4: Effect of pollutants on il5placenta

Parameter	Estimate (s.e)	P-value
Intercept	11.433 (2.475)	<.001
Pbblood	-0.168 (0.056)	0.003
Pbplacenta	375.173 (15.067)	<.001
MBS-sum2	0.396 (0.209)	0.059
PLAT-sum1	1.027 (0.210)	<.001
PLAT-sum2	-1.855 (0.263)	<.001
PLAT-sum3	0.872 (0.364)	0.017
BM-sum1	-1.106 (0.194)	<.001
BM-sum2	0.576 (0.212)	0.007
BM-sum3	-0.705 (0.349)	0.044

Table C2.5: Effect of pollutants on il10placenta

Parameter	Estimate (s.e)	P-value
Intercept	4.168 (1.765)	0.019
Pbblood	-0.076 (0.038)	0.043
Cdplacenta	676.859 (133.326)	<.001
MBS-sum2	0.371 (0.174)	0.034
PLAT-sum1	0.634 (0.179)	<.001
PLAT-sum2	-0.648 (0.223)	0.004
PLAT-sum3	-0.704 (0.294)	0.017
BM-sum1	-0.414 (0.149)	0.006
BM-sum2	-0.301(0.169)	0.074

Table C2.6: Effect of pollutants on tnfplacenta

Parameter	Estimate (s.e)	P-value
Intercept	5.171 (1.355)	<.001
Pbplacenta	60.104 (11.739)	<.001
Cdplacenta	310.259 (130.370)	0.017
MBS-sum1	0.288 (0.132)	0.029
PLAT-sum1	0.451 (0.175)	0.010
PLAT-sum2	-1.017 (0.203)	<.001
PLAT-sum3	0.593 (0.293)	0.043
BM-sum1	0.422 (0.159)	0.009
BM-sum3	-0.659 (0.272)	0.016

Table C2.7: Effect of pollutants on IFNgphacord

Parameter	Estimate (s.e)	P-value
Intercept	915.529 (54.660)	<.001
Pbblood	-13.595 (1.211)	<.001
Cdblood	344.195 (21.041)	<.001
MBS-sum2	10.316 (4.549)	0.023
BM-sum1	-30.037 (4.159)	<.001
BM-sum2	-11.344 (4.400)	0.010
BM-sum3	17.651 (7.406)	0.018

Table C2.8: Effect of pollutants on il4phacord

Parameter	Estimate (s.e)	P-value
Intercept	8.137 (0.917)	<.001
Pbblood	-0.158 (0.021)	<.001
Pbplacenta	59.004 (5.556)	<.001
Cdblood	8.396 (0.352)	<.001
MBS-sum1	-0.326 (0.065)	<.001
BM-sum2	-0.352 (0.067)	<.001

Table C2.9: Effect of pollutants on il5phacord

Parameter	Estimate (s.e)	P-value
Intercept	35.999 (2.542)	<.001
Pbblood	-0.428 (0.059)	<.001
Pbplacenta	-188.578 (15.509)	<.001
Cdblood	1.763 (0.977)	0.072
MBS-sum2	0.557 (0.268)	0.038
MBS-sum3	-0.936 (0.376)	0.013
PLAT-sum2	0.818 (0.271)	0.003
PLAT-sum3	-1.008 (0.343)	0.004
BM-sum1	-1.333 (0.199)	<.001
BM-sum2	-1.349 (0.219)	<.001
BM-sum3	1.983 (0.369)	<.001

Table C2.10: Effect of pollutants on il10phacord

Parameter	Estimate (s.e)	P-value
Intercept	377.553 (33.572)	<.001
Pbblood	-5.856 (0.772)	<.001
Pbplacenta	1178.906 (204.544)	<.001
Cdblood	278.497 (12.907)	<.001
MBS-sum2	-7.463 (3.273)	0.023
MBS-sum3	15.066 (4.667)	0.001
PLAT-sum3	8.425 (3.385)	0.013
BM-sum1	-9.694 (2.591)	<.001
BM-sum3	-19.102 (4.530)	<.001

Table C2.11: Effect of pollutants on il12phacord

Parameter	Estimate (s.e)	P-value
Intercept	327.708 (35.978)	<.001
Pbblood	-5.268 (0.829)	<.001
Pbplacenta	548.055 (218.276)	0.012
Cdblood	96.557 (13.773)	<.001
MBS-sum3	9.965 (4.177)	0.017
PLAT-sum2	29.645 (3.681)	<.001
PLAT-sum3	-11.882 (4.618)	0.010
BM-sum1	11.501 (2.623)	<.001
BM-sum2	-37.676 (2.914)	<.001

Table C2.12: Effect of pollutants on INFgchild

Parameter	Estimate (s.e)	P-value
Intercept	847.305 (93.816)	<.001
Pbblood	-8.058 (2.147)	<.001
Pbplacenta	2647.143 (569.066)	<.001
Cdblood	148.689 (36.003)	<.001
MBS-sum1	23.463 (7.541)	0.002
MBS-sum2	-65.148 (9.446)	<.001
MBS-sum3	41.294 (13.630)	0.003
PLAT-sum1	20.727 (5.591)	<.001

Table C2.13: Effect of pollutants on il4child

Parameter	Estimate (s.e)	P-value
Intercept	10.118 (0.801)	<.001
Pbblood	-0.042 (0.016)	0.010
Pbplacenta	-17.594 (4.332)	<.001
Cdblood	0.887 (0.276)	0.001
Cdplacenta	-95.516 (45.299)	0.036
PLAT-sum2	-0.585 (0.060)	<.001
BM-sum2	0.209 (0.057)	<.001

Table C2.14: Effect of pollutants on il5child

Variable	Estimate (s.e)	P-value
Parameter	318.439 (44.814)	<.001
Pbblood	-8.052 (0.925)	<.001
Pbplacenta	2458.001 (244.074)	<.001
Cdblood	190.809 (15.447)	<.001
Cdplacenta	9984.254 (2553.523)	<.001
MBS-sum2	-24.003 (3.883)	<.001
MBS-sum3	20.860 (5.379)	0.001
PLAT-sum1	-12.436 (2.643)	<.001
BM-sum1	10.711 (2.851)	<.001

Table C2.15: Effect of pollutants on il10child

Parameter	Estimate (s.e)	P-value
Intercept	327.212 (20.918)	<.001
Pbblood	-1.984 (0.480)	<.001
Pbplacenta	-1688.435 (127.322)	<.001
Cdblood	-70.939 (8.044)	<.001
MBS-sum1	11.258 (1.703)	<.001
MBS-sum2	-17.254 (2.111)	<.001
MBS-sum3	6.255 (3.216)	0.052
PLAT-sum3	6.6431 (2.100)	0.002
BM-sum3	-5.499 (2.608)	0.036

Table C2.16: Effect of pollutants on il2child

Parameter	Estimate (s.e)	P-value
Intercept	2794.480 (235.339)	<.001
Pbplacenta	-3718.573 (1296.779)	0.0043
Cdblood	401.972 (84.106)	<.001
Cdplacenta	-1085 (14284)	<.001
MBS-sum1	99.493 (16.183)	<.001
MBS-sum2	-96.678 (21.312)	<.001
PLAT-sum1	84.494 (16.845)	<.001
PLAT-sum2	-71.089 (21.380)	0.001
BM-sum2	49.067 (17.566)	0.005

Appendix C3: Areas under curves from multivariate logistic models

Table C3.1: Area under curve (AUC), STD and 95% (C.I.)

	With Phadinhalant			Without Phadinhalant		
Allergic outcome	AUC	STD	95% (C.I.)	AUC	STD	95% (C.I.)
Atopic eczema	0.719	0.034	(0.652, .787)	0.686	0.035	(0.617, 0.755)
Otherdermatitis	0.520	0.067	(0.387, .653)	0.593	0.068	(0.457, 0.730)
PhadCHinhalant	0.697	0.112	(0.473, .921)	-	-	-
fx5CHfood	0.138	0.030	(0.078, .199)	0.631	0.072	(0.487, 0.774)

Table C3.2: Area under curve (AUC), STD and 95% (C.I.)

	With PhadCHinhalant			Without PhadCHinhalant		
Allergic outcome	AUC	STD	95% (C.I.)	AUC	STD	95% (C.I.)
Atopic eczema	0.629	0.035	(0.558, 0.699)	0.611	0.036	(0.540, 0.682)
Otherdermatitis	0.516	0.067	(0.383, 0.649)	0.730	0.065	(0.599, 0.861)
PhadCHinhalant	0.338	0.091	(0.156, 0.520)	-	-	-
fx5CHfood	0.672	0.071	(0.529, 0.814)	0.469	0.068	(0.333, 0.605)

Table C3.3: Area under curve (AUC), STD and 95% (C.I.)

	With Phadinhalant			Without Phadinhalant		
Allergic outcome	AUC	STD	95% (C.I.)	AUC	STD	95% (C.I.)
Atopic eczema	0.669	0.035	(0.599, 0.739)	0.704	0.034	(0.636, 0.772)
Otherdermatitis	0.649	0.068	(0.513, 0.785)	0.630	0.068	(0.494, 0.766)
PhadCHinhalant	0.317	0.087	(0.142, 0.492)	-	-	-
fx5CHfood	0.739	0.068	(0.603, 0.875)	0.545	0.071	(0.403, 0.687)

Appendix D: Diagnostic residual plots

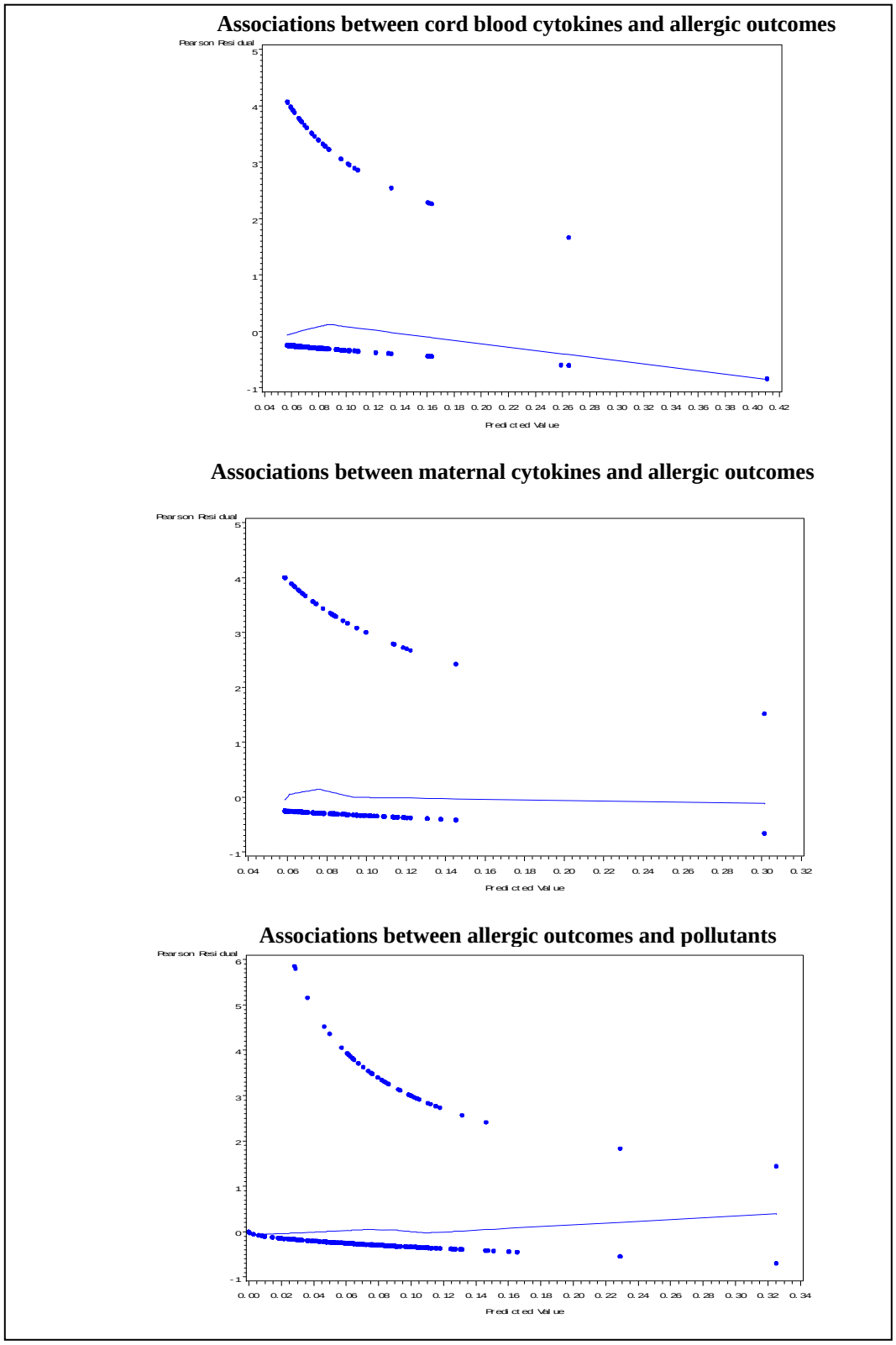

Figure D1: Panel of plots of Pearson residuals against predicted probabilities

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Datum: **27.08.2007**